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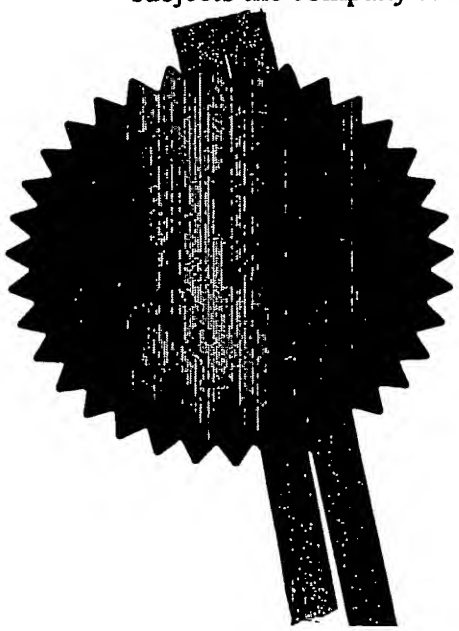

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Patents ADP number *(if you know it)*	06275622002
If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND
4. Title of the invention	PROTEINS
5. Name of your agent *(if you have one)*	Carpmaels & Ransford
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Signature Carpmaels & Ransford Date **23rd December 2002**

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Proteins

This invention relates to novel proteins, termed INSP005a and INSP005b, herein identified as secreted proteins, in particular members of the metalloprotease family and to the use of these proteins and nucleic acid sequences from the encoding genes in the diagnosis, prevention and treatment of disease.

All publications, patents and patent applications cited herein are incorporated in full by reference.

Background

The process of drug discovery is presently undergoing a fundamental revolution as the era of functional genomics comes of age. The term "functional genomics" applies to an approach utilising bioinformatics tools to ascribe function to protein sequences of interest. Such tools are becoming increasingly necessary as the speed of generation of sequence data is rapidly outpacing the ability of research laboratories to assign functions to these protein sequences.

As bioinformatics tools increase in potency and in accuracy, these tools are rapidly replacing the conventional techniques of biochemical characterisation. Indeed, the advanced bioinformatics tools used in identifying the present invention are now capable of outputting results in which a high degree of confidence can be placed.

Various institutions and commercial organisations are examining sequence data as they become available and significant discoveries are being made on an on-going basis. However, there remains a continuing need to identify and characterise further genes and the polypeptides that they encode, as targets for research and for drug discovery.

Secreted protein background

The ability for cells to make and secrete extracellular proteins is central to many biological processes. Enzymes, growth factors, extracellular matrix proteins and signalling molecules are all secreted by cells. This is through fusion of a secretory vesicle with the plasma membrane. In most cases, but not all, proteins are directed to the endoplasmic reticulum and into secretory vesicles by a signal peptide. Signal peptides are cis-acting sequences that affect the transport of polypeptide chains from the cytoplasm to a membrane bound compartment such as a secretory vesicle. Polypeptides that are targeted to the secretory

vesicles are either secreted into the extracellular matrix or are retained in the plasma membrane. The polypeptides that are retained in the plasma membrane will have one or more transmembrane domains. Examples of secreted proteins that play a central role in the functioning of a cell are cytokines, hormones, extracellular matrix proteins (adhesion molecules), proteases, and growth and differentiation factors. Description of some of the properties of these proteins follows.

Proteases are enzymes that irreversibly hydrolyse amide bonds in peptides and proteins. Proteases are widely distributed and are involved in many different biological processes, from activation of proteins and peptides to degradation of proteins. Despite the fact that proteases have been shown to be involved in many different diseases, drugs targeted to proteases are still rare in pharmacy, although inhibitors of angiotensin converting enzyme (ACE) have been among the most successful antihypertensive drugs for several years. Proteases have recently received substantial publicity as valuable therapeutic targets following the approval of HIV protease inhibitors.

Proteases can be divided in large Families. The term "Family" is used to describe a group of proteases in which each member shows an evolutionary relationship to at least one other member, either throughout the whole sequence or at least in the part of the sequence responsible for catalytic activity. The name of each Family reflects the catalytic activity type of the proteases in the Family. Thus, serine proteases belong to the S family, threonine proteases belong to the T family, aspartyl proteases belong to the A family, cysteine proteases belong to the C family and metalloproteinases belong to the M family. Metalloproteases and Serine proteases are commonly found in the extracellular matrix.

Metalloproteases (M family):

Metalloproteases can be divided in 2 major groups depending on the presence or absence of a the Zinc binding motif (HEXXH).

1.1 Presence of HEXXH motif (22 families): Prosite number: PDOC00129

Families with interesting members:

M2: Peptidyl-dipeptidase A (Angiotensin I Coverting Enzyme: ACE)

M13: Neprilysin (Enkephalinase A=neutal endopeptidase=NEP), Endothelial Converting Enzyme (ECE)

M10B: Matrixin (Matrix Metalloproteases=MMPs)

M12B: Reprolysin (ADAM-10; ADAM-17= TNF-alpha Converting Enzyme = TACE)/Desintegrin (other ADAM proteases). The ADAMs are a large, widely expressed and developmentally regulated family of proteins with multiple potential functions in cell-cell and cell-matrix interactions. Among them TACE represents a new emerging target for arthritis disease.

M41: This family contains ATP-dependent metalloproteases: FtsH, proteasome proteins.

One of the largest therapeutically interesting group of metalloproteinases is the Matrix Metalloproteinases family (MMPs). Matrix metalloproteinases are a family of Zinc containing enzymes that are responsible for the remodeling of extracellular matrix throughout the body. They have been shown to be involved in cancer (increase invasiveness, effects on new blood vessel), and in arthritis (involvement in cartilage degradation (Dahlberg, L., *et al.,* Arthritis Rheum. 2000 43(3):673-82) and also TNF-alpha conversion (Hanemaaijer, R., *et al.,* J Biol Chem. 1997 272(50):31504-9, Shlopov, B.V., *et al.,*. Arthritis Rheum. 1997 40(11):2065-74)). Indeed, different MMPs have been shown to be overexpressed in diseases such as arthritis (Seitz, M., *et al.,* Rheumatology (Oxford). 2000 39(6):637-645, Yoshihara, Y., *et al.,* Ann Rheum Dis. 2000 59(6):455-61, Yamanaka, H., *et al.,* Lab Invest. 2000 80(5):677-87, Jovanovic, D.V., *et al.,* Arthritis Rheum. 2000 May;43(5):1134-44, Ribbens, C., *et al.*, J Rheumatol. 2000 27(4):888-93) and cancer (Sakamoto, Y., *et al.*, Int J Oncol. 2000 17(2):237-43, Kerkela, E., *et al.,* J Invest Dermatol. 2000 114(6):1113-9, Fang, J., *et al.*, Proc Natl Acad Sci U S A. 2000 97(8):3884-9, Sun, Y., *et al.*, J Biol Chem. 2000 275(15):11327-32, McCawley, L.J., *et al.*, Mol Med Today. 2000 6(4):149-56, Ara,T., *et al.,* J Pediatr Surg. 2000 35(3):432-7, Shigemasa, K., *et al.,* Med Oncol. 2000 17(1):52-8, Nakanishi, K., *et al.,* Hum Pathol. 2000 31(2):193-200, Dalberg, K., *et al.*, World J Surg. 2000 24(3):334-40). Inhibitors of these enzymes have been suggested as potential therapeutic agents for the use in the treatment of both cancer and arthritis. More recently it has been shown that MMPs may also have a role in the release of soluble cytokine receptors, growth factors and other cell

mediators, suggesting that selective MMPs inhibitors may have wider therapeutic applications than previously proposed.

MMPs have been divided in 4 families based on amino-acid sequence homologies of their domain structure, other than the catalytic region.

Minimal domain family: matrilysin (PUMP-1, MMP-7) cleaves proteoglycan, laminin and fibronectin

Hemopexin domain family:

Collagenases: unique ability to cleave fibrillar collagen. The role of collagenases in cartilage degradation , make them attractive targets for the treatment of rheumatoid and osteo-arthritis.

- collagenases fibroblast collagenase (interstitial collagenase, MMP-1)
- neutrophil collagenase (MMP-8)
- collagenase-3 (MMP-13)

Metalloelastase: MME (MMP-12)

Stromelysin-1 (MMP-3), 2 (MMP-10) and 3 (MMP-11). MMP-11 is excreted as an active form and it's function could be to activate other MMPs.

Fibronectin domain family: degrades a large number of matrix substrates (gelatin, elastin, type IV collagen)

Gelatinase A (MMP-2); beside it's involvement in cancer (tumor invasivness), it is proposed as a potential target for the discovery of antiplatelet agent as it may play an important role in platelet activation.

Gelatinase B (MMP-9)

Transmembrane domain family:

MT-1-MMP, MT-4-MMP, MMP-14, MMP-17

A lot of studies concerning the different specificities of MMPs and their relative involvement in some diseases are on going.

1.2 Absence of HEXXH motifs (18 families):

Families with interesting members:

M24A: Methionyl aminopeptidase, type 1 (including procaryotic and eucaryotic MAP-1) / Prosite number: PDOC00575

M24C: Methionyl aminopeptidase, type 2 (including eucaryotic MAP-2) / Prosite number: PDOC00575

Table 1. Summary of metalloproteases and their function

Protease name	EC number	Biological function	Disease associated	Regulation
MMP-12	3.4.24.65	MMPs function; elastin degradation; process TNF-alpha;convert plasminogen to angiotensin	involvement in lung disorders, emphysema, cystic fibrosis	enhanced expression in some skin diseases
MMP-2	3.4.24.24	MMPs function	cancer	overexpression in colorectal cancer
ADAM-12	3.4.24	cell-cell, cell-matrix interaction		up-regulated in several human carcinomas
TACE	3.4.24.?	Processing of the membrane bound TNF-alphaand other cell bound molecule	inflammation, rheumatoid arthritis, neuroimmunological diseases	up-regulated in arthritis affected cartilage
ACE	3.4.15.1	production of angiotensin II	hypertension	
ECE-1	3.4.24.71	process the precursor of the vasoconstrictor endothelin	cardiovascular	
NEP	3.4.24.11	cleaves neuropeptides, hormones and immune	cardiovascular, arthritis (?)	

		mediator		
FtsH	?	protein secretion, assembly, degradation, cell cycle, stress response	bacterial infections	-
Deformylase	3.5.1.31	removes the formyl group from N-terminal from newly synthesized proteins	bacterial infections	-
Proteasome	3.4.99.46	protein degradation, antigen presentation	cancer	

Metalloproteases are implicated across a wide variety of therapeutic areas. These include respiratory diseases (Segura-Valdez, L., *et al.*, Chest. 2000 117(3):684-94, Tanaka, H., *et al.*, J Allergy Clin Immunol. 2000 105(5):900-5, Hoshino, M., *et al.*, J Allergy Clin Immunol. 1999 104(2 Pt 1):356-63, Mautino, G., *et al.*, Am J Respir Crit Care Med. 1999 160(1):324-30, Dalal, S., *et al.*, Chest. 2000 117(5 Suppl 1):227S-8S, Ohnishi, K., *et al.*, Lab Invest. 1998 78(9):1077-87), cardiovascular disease (Taniyama, Y., *et al.*, Circulation. 2000 102(2):246-52, Hong, B.K., *et al.*, Yonsei Med J. 2000 41(1):82-8, Galis, Z.S., *et al.*, Proc Natl Acad Sci U S A. 1995 92(2):402-6), bacterial infections (Scozzafava, A., *et al.*, J Med Chem. 2000 43(9):1858-65, Vencill, C.F., *et al.*, Biochemistry. 1985 24(13):3149-57, Steinbrink, D.R, *et al.*, J Biol Chem. 1985 260(5):2771-6, Lopez-Boado, Y.S., *et al.*, J Cell Biol. 2000 148(6):1305-15, Chang, J.C., *et al.*, Thorax. 1996 51(3):306-11, Dammann, T., *et al.*, Mol. Microbiol. 6:2267-2278(1992), Wassif, C., *et al.*, J. Bacteriol. 177 (20), 5790-5798 (1995), oncology (Sakamoto, Y., *et al.*, Int J Oncol. 2000 17(2):237-43, Kerkela, E., *et al.*, J Invest Dermatol. 2000 114(6):1113-9, Fang, J., *et al.*, Proc Natl Acad Sci U S A. 2000 97(8):3884-9, Sun, Y., *et al.*, J Biol Chem. 2000 275(15):11327-32, McCawley, L.J., *et al.*, Mol Med Today. 2000 6(4):149-56, Ara,T., *et al.*, J Pediatr Surg. 2000 35(3):432-7, Shigemasa, K., *et al.*, Med Oncol. 2000 17(1):52-8, Nakanishi, K., *et al.*, Hum Pathol. 2000 31(2):193-200, Dalberg, K., *et al.*, World J Surg. 2000 24(3):334-40), and inflammation (rheumatoid and osteo-arthritis (Ribbens, C., *et al.*, J Rheumatol. 2000 27(4):888-93, Kageyama, Y., *et al.*, Clin Rheumatol. 2000 19(1):14-20, Shlopov, B.V., *et al.*, Arthritis Rheum. 2000 Jan;43(1):195-205)).

Metalloproteases are also implicated in the physiology and pathology of sexual reproduction, and have been implicated in therapies associated with modulating chorion status, the zona reaction, the formation of fertilisation membranes, contraception and infertility (Shibata *et al.* (2000) J.Biol.Chem vol.275, No.12 p8349)

Accordingly, identification of novel metalloproteases is of extreme importance in increasing understanding of the underlying pathways that lead to certain disease states in which these proteins are implicated, and in developing more effective gene or drug therapies to treat these disorders.

THE INVENTION

The invention is based on the discovery that the INSP005a and INSP005b proteins function as secreted protease molecules and moreover as secreted protease molecules of the metalloprotease family. Preferably, the INSP005a and INSP005b proteins are members of the choriolysin/astacin-like family of metalloproteases.

In one embodiment of the first aspect of the invention, there is provided a polypeptide which:

(i) comprises the amino acid sequence as recited in SEQ ID NO:14;

(ii) is a fragment thereof having function as a secreted protein of the metalloprotease class or having an antigenic determinant in common with the polypeptides of (i); or

(iii) is a functional equivalent of (i) or (ii).

Preferably, a polypeptide according to this embodiment consists of the sequence recited in SEQ ID NO:14. The polypeptide having the sequence recited in SEQ ID NO:14 is referred to hereafter as "the INSP005a polypeptide".

In a second embodiment of the first aspect of the invention, there is provided a polypeptide which:

(i) comprises the amino acid sequence as recited in SEQ ID NO:34 or SEQ ID NO:36;

(ii) is a fragment thereof having function as a secreted protein of the metalloprotease class or having an antigenic determinant in common with the polypeptides of (i); or

(iii) is a functional equivalent of (i) or (ii).

Preferably, a polypeptide according to this embodiment consists of the sequence recited in

SEQ ID NO:34 or SEQ ID NO:36. The polypeptide having the sequence recited in SEQ ID NO:34 is referred to hereafter as "the INSP005b polypeptide".

Although the Applicant does not wish to be bound by this theory, it is postulated that the first 23 amino acids of the INSP005b polypeptide form a signal peptide. The nucleotide sequence encoding the postulated INSP005b mature polypeptide, and the amino acid sequence of the INSP005b mature polypeptide, are recited in SEQ ID NO:35 and SEQ ID NO:36, respectively. The polypeptide having the sequence recited in SEQ ID NO:36 is referred to hereafter as "the INSP005b mature polypeptide".

Preferably, a polypeptide according to the above-described aspects of the invention functions as a metalloprotease. The term "metalloprotease" is well understood in the art and the skilled worker will readily be able to ascertain metalloprotease activity using one of a variety of assays known in the art. For example, two commonly-applied assays are the quantitative [^{3}H] gelatin assay (Martin *et al.*, Kidney Int. 36, 790-801) and the gelatin zymography assay (Herron G.S. *et al.*, J. Biol. Chem. 1986, 261, 2814-2818).

More preferably, a polypeptide according to the above-described aspects of the invention is a member of the choriolysin/astacin-like family of metalloproteases.

The INSP005a polypeptides, INSP005b polypeptides and the INSP005b mature polypeptides are referred to herein as "the INSP005 polypeptides".

In a second aspect, the invention provides a purified nucleic acid molecule which encodes a polypeptide of the first aspect of the invention. Preferably, the purified nucleic acid molecule has the nucleic acid sequence as recited in SEQ ID NO:13 (encoding the INSP005a polypeptide), SEQ ID NO:33 (encoding the INSP005b polypeptide) or SEQ ID NO:35 (encoding the INSP005b mature polypeptide), or is a redundant equivalent or fragment of either of these sequences.

In a third aspect, the invention provides a purified nucleic acid molecule which hydridizes under high stringency conditions with a nucleic acid molecule of the second aspect of the invention.

In a fourth aspect, the invention provides a vector, such as an expression vector, that contains a nucleic acid molecule of the second or third aspect of the invention. In a preferred embodiment of this aspect of the invention the vector is the PCR-TOPO-IPAAA78836-1 vector (see Figure 9 and SEQ ID NO:38). In a further preferred

embodiment of this aspect of the invention the vector is the PCR-TOPO-IPAAA78836-2 vector (see Figure 12 and SEQ ID NO:39).

In a fifth aspect, the invention provides a host cell transformed with a vector of the fourth aspect of the invention.

In a sixth aspect, the invention provides a ligand which binds specifically to, and which preferably inhibits the metalloprotease activity of a polypeptide of the first aspect of the invention. Ligands to a polypeptide according to the invention may come in various forms, including natural or modified substrates, enzymes, receptors, small organic molecules such as small natural or synthetic organic molecules of up to 2000Da, preferably 800Da or less, peptidomimetics, inorganic molecules, peptides, polypeptides, antibodies, structural or functional mimetics of the aforementioned.

In a seventh aspect, the invention provides a compound that is effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

A compound of the seventh aspect of the invention may either increase (agonise) or decrease (antagonise) the level of expression of the gene or the activity of the polypeptide. Importantly, the identification of the function of the INSP005 polypeptides allows for the design of screening methods capable of identifying compounds that are effective in the treatment and/or diagnosis of disease. Ligands and compounds according to the sixth and seventh aspects of the invention may be identified using such methods. These methods are included as aspects of the present invention.

In an eighth aspect, the invention provides a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in therapy or diagnosis of diseases in which metalloproteases are implicated. These molecules may also be used in the manufacture of a medicament for the treatment of such diseases, particularly respiratory disorders, including emphysema and cystic fibrosis, metabolic disorders, cardiovascular disorders, bacterial infections, hypertension, proliferative disorders, including cancer, autoimmune/inflammatory disorders, including rheumatoid arthritis, neurological

disorders, developmental disorders and reproductive disorders.

In a ninth aspect, the invention provides a method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide of the first aspect of the invention or the activity of a polypeptide of the first aspect of the invention in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of disease. Such a method will preferably be carried out *in vitro*. Similar methods may be used for monitoring the therapeutic treatment of disease in a patient, wherein altering the level of expression or activity of a polypeptide or nucleic acid molecule over the period of time towards a control level is indicative of regression of disease.

A preferred method for detecting polypeptides of the first aspect of the invention comprises the steps of: (a) contacting a ligand, such as an antibody, of the sixth aspect of the invention with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

A number of different such methods according to the ninth aspect of the invention exist, as the skilled reader will be aware, such as methods of nucleic acid hybridization with short probes, point mutation analysis, polymerase chain reaction (PCR) amplification and methods using antibodies to detect aberrant protein levels. Similar methods may be used on a short or long term basis to allow therapeutic treatment of a disease to be monitored in a patient. The invention also provides kits that are useful in these methods for diagnosing disease.

In a tenth aspect, the invention provides for the use of a polypeptide of the first aspect of the invention as a secreted protein, preferably as a metalloprotease.

In an eleventh aspect, the invention provides a pharmaceutical composition comprising a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, in conjunction with a pharmaceutically-acceptable carrier.

In a twelfth aspect, the present invention provides a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a

vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in the manufacture of a medicament for the diagnosis or treatment of a disease, such as respiratory disorders, including emphysema and cystic fibrosis, metabolic disorders, cardiovascular disorders, bacterial infection, hypertension, proliferative disorders, including cancer, autoimmune/inflammatory disorders, including rheumatoid arthritis, neurological disorders, developmental disorders, reproductive disorders or other diseases in which metalloproteases are implicated.

In a thirteenth aspect, the invention provides a method of treating a disease in a patient comprising administering to the patient a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention.

For diseases in which the expression of a natural gene encoding a polypeptide of the first aspect of the invention, or in which the activity of a polypeptide of the first aspect of the invention, is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an agonist. Conversely, for diseases in which the expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an antagonist. Examples of such antagonists include antisense nucleic acid molecules, ribozymes and ligands, such as antibodies.

In a fourteenth aspect, the invention provides transgenic or knockout non-human animals that have been transformed to express higher, lower or absent levels of a polypeptide of the first aspect of the invention. Such transgenic animals are very useful models for the study of disease and may also be used in screening regimes for the identification of compounds that are effective in the treatment or diagnosis of such a disease.

A summary of standard techniques and procedures which may be employed in order to utilise the invention is given below. It will be understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors and reagents described. It is also to be understood that the terminology used herein is for the purpose of describing

particular embodiments only and it is not intended that this terminology should limit the scope of the present invention. The extent of the invention is limited only by the terms of the appended claims.

Standard abbreviations for nucleotides and amino acids are used in this specification.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology, which are within the skill of those working in the art.

Such techniques are explained fully in the literature. Examples of particularly suitable texts for consultation include the following: Sambrook Molecular Cloning; A Laboratory Manual, Second Edition (1989); DNA Cloning, Volumes I and II (D.N Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); Transcription and Translation (B.D. Hames & S.J. Higgins eds. 1984); Animal Cell Culture (R.I. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984); the Methods in Enzymology series (Academic Press, Inc.), especially volumes 154 & 155; Gene Transfer Vectors for Mammalian Cells (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Immunochemical Methods in Cell and Molecular Biology (Mayer and Walker, eds. 1987, Academic Press, London); Scopes, (1987) Protein Purification: Principles and Practice, Second Edition (Springer Verlag, N.Y.); and Handbook of Experimental Immunology, Volumes I-IV (D.M. Weir and C. C. Blackwell eds. 1986).

As used herein, the term "polypeptide" includes any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e. peptide isosteres. This term refers both to short chains (peptides and oligopeptides) and to longer chains (proteins).

As described above, the polypeptide of the present invention may be in the form of a mature protein or may be a pre-, pro- or prepro- protein that can be activated by cleavage of the pre-, pro- or prepro- portion to produce an active mature polypeptide. In such polypeptides, the pre-, pro- or prepro- sequence may be a leader or secretory sequence or may be a sequence that is employed for purification of the mature polypeptide sequence.

The polypeptide of the first aspect of the invention may form part of a fusion protein. For example, it is often advantageous to include one or more additional amino acid sequences

which may contain secretory or leader sequences, pro-sequences, sequences which aid in purification, or sequences that confer higher protein stability, for example during recombinant production. Alternatively or additionally, the mature polypeptide may be fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol).

Polypeptides may contain amino acids other than the 20 gene-encoded amino acids, modified either by natural processes, such as by post-translational processing or by chemical modification techniques which are well known in the art. Among the known modifications which may commonly be present in polypeptides of the present invention are glycosylation, lipid attachment, sulphation, gamma-carboxylation, for instance of glutamic acid residues, hydroxylation and ADP-ribosylation. Other potential modifications include acetylation, acylation, amidation, covalent attachment of flavin, covalent attachment of a haeme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulphide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, GPI anchor formation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl terminus in a polypeptide, or both, by a covalent modification is common in naturally-occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention.

The modifications that occur in a polypeptide often will be a function of how the polypeptide is made. For polypeptides that are made recombinantly, the nature and extent of the modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the polypeptide in question. For instance, glycosylation patterns vary between different types of host cell.

The polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include, where the polypeptide is a naturally occurring polypeptide, isolated

naturally-occurring polypeptides (for example purified from cell culture), recombinantly-produced polypeptides (including fusion proteins), synthetically-produced polypeptides or polypeptides that are produced by a combination of these methods. The term "isolated" does not denote the method by which the polypeptide is obtained or the level of purity of the preparation. Thus, such isolated species may be produced recombinantly, isolated directly from the cell or tissue of interest or produced synthetically based on the determined sequences.

The functionally-equivalent polypeptides of the first aspect of the invention may be polypeptides that are homologous to the INSP005 polypeptides. Two polypeptides are said to be "homologous", as the term is used herein, if the sequence of one of the polypeptides has a high enough degree of identity or similarity to the sequence of the other polypeptide. "Identity" indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity" indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

Homologous polypeptides therefore include natural biological variants (for example, allelic variants or geographical variations within the species from which the polypeptides are derived) and mutants (such as mutants containing amino acid substitutions, insertions or deletions) of the INSP005 polypeptides. Such mutants may include polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg; or among the aromatic residues Phe and Tyr. Particularly preferred are variants in which several, i.e. between 5 and 10, 1 and 5, 1 and 3, 1 and 2 or just 1 amino acids are substituted, deleted or added in any combination. Especially preferred are silent

substitutions, additions and deletions, which do not alter the properties and activities of the protein. Also especially preferred in this regard are conservative substitutions.

Such mutants also include polypeptides in which one or more of the amino acid residues includes a substituent group;

Typically, greater than 30% identity between two polypeptides is considered to be an indication of functional equivalence. Preferably, functionally equivalent polypeptides of the first aspect of the invention have a degree of sequence identity with the INSP005 polypeptides, or with active fragments thereof, of greater than 80%. More preferred polypeptides have degrees of identity of greater than 85%, 90%, 95%, 98%, 99% or more, respectively.

The functionally-equivalent polypeptides of the first aspect of the invention may also be polypeptides which have been identified using one or more techniques of structural alignment. For example, the Inpharmatica Genome Threader technology that forms one aspect of the search tools used to generate the Biopendium search database may be used (see co-pending PCT patent application PCT/GB01/01105) to identify polypeptides of presently-unknown function which, while having low sequence identity as compared to the INSP005 polypeptides, are predicted to have secreted molecule activity, by virtue of sharing significant structural homology with the INSP005 polypeptide sequences. By "significant structural homology" is meant that the Inpharmatica Genome Threader predicts two proteins to share structural homology with a certainty of 10% and above.

The polypeptides of the first aspect of the invention also include fragments of the INSP005 polypeptides and fragments of the functional equivalents of the INSP005 polypeptides, provided that those fragments retain metalloprotease activity or have an antigenic determinant in common with the INSP005 polypeptides.

As used herein, the term "fragment" refers to a polypeptide having an amino acid sequence that is the same as part, but not all, of the amino acid sequence of the INSP005 polypeptides or one of its functional equivalents. The fragments should comprise at least n consecutive amino acids from the sequence and, depending on the particular sequence, n preferably is 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more). Small fragments may form an antigenic determinant.

Such fragments may be "free-standing", i.e. not part of or fused to other amino acids or

polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the fragment of the invention most preferably forms a single continuous region. For instance, certain preferred embodiments relate to a fragment having a pre - and/or pro- polypeptide region fused to the amino terminus of the fragment and/or an additional region fused to the carboxyl terminus of the fragment. However, several fragments may be comprised within a single larger polypeptide.

The polypeptides of the present invention or their immunogenic fragments (comprising at least one antigenic determinant) can be used to generate ligands, such as polyclonal or monoclonal antibodies, that are immunospecific for the polypeptides. Such antibodies may be employed to isolate or to identify clones expressing the polypeptides of the invention or to purify the polypeptides by affinity chromatography. The antibodies may also be employed as diagnostic or therapeutic aids, amongst other applications, as will be apparent to the skilled reader.

The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art. As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')2 and Fv, which are capable of binding to the antigenic determinant in question. Such antibodies thus bind to the polypeptides of the first aspect of the invention.

By "substantially greater affinity" we mean that there is a measurable increase in the affinity for a polypeptide of the invention as compared with the affinity for known secreted proteins.

Preferably, the affinity is at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10^3-fold, 10^4-fold, 10^5-fold or 10^6-fold greater for a polypeptide of the invention than for known secreted proteins.

If polyclonal antibodies are desired, a selected mammal, such as a mouse, rabbit, goat or horse, may be immunised with a polypeptide of the first aspect of the invention. The polypeptide used to immunise the animal can be derived by recombinant DNA technology or can be synthesized chemically. If desired, the polypeptide can be conjugated to a carrier protein. Commonly used carriers to which the polypeptides may be chemically coupled include bovine serum albumin, thyroglobulin and keyhole limpet haemocyanin. The

coupled polypeptide is then used to immunise the animal. Serum from the immunised animal is collected and treated according to known procedures, for example by immunoaffinity chromatography.

Monoclonal antibodies to the polypeptides of the first aspect of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies using hybridoma technology is well known (see, for example, Kohler, G. and Milstein, C., Nature 256: 495-497 (1975); Kozbor *et al.*, Immunology Today 4: 72 (1983); Cole *et al.*, 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985).

Panels of monoclonal antibodies produced against the polypeptides of the first aspect of the invention can be screened for various properties, i.e., for isotype, epitope, affinity, etc. Monoclonal antibodies are particularly useful in purification of the individual polypeptides against which they are directed. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance by PCR techniques known in the art, and cloned and expressed in appropriate vectors.

Chimeric antibodies, in which non-human variable regions are joined or fused to human constant regions (see, for example, Liu *et al.*, Proc. Natl. Acad. Sci. USA, 84, 3439 (1987)), may also be of use.

The antibody may be modified to make it less immunogenic in an individual, for example by humanisation (see Jones *et al.*, Nature, 321, 522 (1986); Verhoeyen *et al.*, Science, 239, 1534 (1988); Kabat *et al.*, J. Immunol., 147, 1709 (1991); Queen *et al.*, Proc. Natl Acad. Sci. USA, 86, 10029 (1989); Gorman *et al.*, Proc. Natl Acad. Sci. USA, 88, 34181 (1991); and Hodgson *et al.*, Bio/Technology, 9, 421 (1991)). The term "humanised antibody", as used herein, refers to antibody molecules in which the CDR amino acids and selected other amino acids in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted in place of the equivalent amino acids in a human antibody. The humanised antibody thus closely resembles a human antibody but has the binding ability of the donor antibody.

In a further alternative, the antibody may be a "bispecific" antibody, that is an antibody having two different antigen binding domains, each domain being directed against a different epitope.

Phage display technology may be utilised to select genes which encode antibodies with binding activities towards the polypeptides of the invention either from repertoires of PCR amplified V-genes of lymphocytes from humans screened for possessing the relevant antibodies, or from naive libraries (McCafferty, J. *et al.*, (1990), Nature 348, 552-554; Marks, J. *et al.*, (1992) Biotechnology 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. *et al.*, (1991) Nature 352, 624-628).

Antibodies generated by the above techniques, whether polyclonal or monoclonal, have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme.

Preferred nucleic acid molecules of the second and third aspects of the invention are those which encode the polypeptide sequences recited in SEQ ID NO:14, SEQ ID NO:34, or SEQ ID NO:36 and functionally equivalent polypeptides. These nucleic acid molecules may be used in the methods and applications described herein. The nucleic acid molecules of the invention preferably comprise at least n consecutive nucleotides from the sequences disclosed herein where, depending on the particular sequence, n is 10 or more (for example, 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

The nucleic acid molecules of the invention also include sequences that are complementary to nucleic acid molecules described above (for example, for antisense or probing purposes).

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance cDNA, synthetic DNA or genomic DNA. Such nucleic acid molecules may be obtained by cloning, by chemical synthetic techniques or by a combination thereof. The nucleic acid molecules can be prepared, for example, by chemical synthesis using techniques such as solid phase phosphoramidite chemical synthesis, from genomic or cDNA libraries or by separation from an organism. RNA molecules may generally be generated by the *in vitro* or *in vivo* transcription of DNA sequences.

The nucleic acid molecules may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The term "nucleic acid molecule" also includes analogues of DNA and RNA, such as those containing modified backbones, and peptide nucleic acids (PNA). The term "PNA", as used herein, refers to an antisense molecule or an anti-gene agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues, which preferably ends in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan in a cell, where they preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. *et al.* (1993) Anticancer Drug Des. 8:53-63).

A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:14 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:13. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:34 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:33. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:36 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:35.

These molecules also may have a different sequence which, as a result of the degeneracy of the genetic code, encodes a polypeptide of SEQ ID NO:14, SEQ ID NO:34 or SEQ ID NO:36. Such nucleic acid molecules may include, but are not limited to, the coding sequence for the mature polypeptide by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pro-, pre- or prepro- polypeptide sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with further additional, non-coding sequences, including non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals), ribosome binding and mRNA stability. The nucleic acid molecules may also include additional sequences which encode additional amino acids, such as those which provide additional functionalities.

The nucleic acid molecules of the second and third aspects of the invention may also encode the fragments or the functional equivalents of the polypeptides and fragments of the first aspect of the invention. Such a nucleic acid molecule may be a naturally-occurring variant such as a naturally-occurring allelic variant, or the molecule may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid

molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or insertions. The substitutions, deletions or insertions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or insertions.

The nucleic acid molecules of the invention can also be engineered, using methods generally known in the art, for a variety of reasons, including modifying the cloning, processing, and/or expression of the gene product (the polypeptide). DNA shuffling by random fragmentation and PCR re-assembly of gene fragments and synthetic oligonucleotides are included as techniques which may be used to engineer the nucleotide sequences. Site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations and so forth.

Nucleic acid molecules which encode a polypeptide of the first aspect of the invention may be ligated to a heterologous sequence so that the combined nucleic acid molecule encodes a fusion protein. Such combined nucleic acid molecules are included within the second or third aspects of the invention. For example, to screen peptide libraries for inhibitors of the activity of the polypeptide, it may be useful to express, using such a combined nucleic acid molecule, a fusion protein that can be recognised by a commercially-available antibody. A fusion protein may also be engineered to contain a cleavage site located between the sequence of the polypeptide of the invention and the sequence of a heterologous protein so that the polypeptide may be cleaved and purified away from the heterologous protein.

The nucleic acid molecules of the invention also include antisense molecules that are partially complementary to nucleic acid molecules encoding polypeptides of the present invention and that therefore hybridize to the encoding nucleic acid molecules (hybridization). Such antisense molecules, such as oligonucleotides, can be designed to recognise, specifically bind to and prevent transcription of a target nucleic acid encoding a polypeptide of the invention, as will be known by those of ordinary skill in the art (see, for example, Cohen, J.S., Trends in Pharm. Sci., 10, 435 (1989), Okano, J. Neurochem. 56, 560 (1991); O'Connor, J. Neurochem 56, 560 (1991); Lee *et al.*, Nucleic Acids Res 6, 3073 (1979); Cooney *et al.*, Science 241, 456 (1988); Dervan *et al.*, Science 251, 1360 (1991).

The term "hybridization" as used here refers to the association of two nucleic acid molecules with one another by hydrogen bonding. Typically, one molecule will be fixed to a solid support and the other will be free in solution. Then, the two molecules may be placed in contact with one another under conditions that favour hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase molecule to the solid support (Denhardt's reagent or BLOTTO); the concentration of the molecules; use of compounds to increase the rate of association of molecules (dextran sulphate or polyethylene glycol); and the stringency of the washing conditions following hybridization (see Sambrook *et al.* [*supra*]).

The inhibition of hybridization of a completely complementary molecule to a target molecule may be examined using a hybridization assay, as known in the art (see, for example, Sambrook *et al* [*supra*]). A substantially homologous molecule will then compete for and inhibit the binding of a completely homologous molecule to the target molecule under various conditions of stringency, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511).

"Stringency" refers to conditions in a hybridization reaction that favour the association of very similar molecules over association of molecules that differ. High stringency hybridisation conditions are defined as overnight incubation at 42(C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at approximately 65(C. Low stringency conditions involve the hybridisation reaction being carried out at 35(C (see Sambrook *et al.* [*supra*]). Preferably, the conditions used for hybridization are those of high stringency.

Preferred embodiments of this aspect of the invention are nucleic acid molecules that are at least 70% identical over their entire length to nucleic acid molecules encoding the INSP005 polypeptides (SEQ ID NO:13, SEQ ID NO:33 and SEQ ID NO:35), and nucleic acid molecules that are substantially complementary to such nucleic acid molecules. Preferably, a nucleic acid molecule according to this aspect of the invention comprises a region that is at least 80% identical over its entire length to the nucleic acid molecule

having the sequence given in SEQ ID NO:13, SEQ ID NO:33 or SEQ ID NO:35 or a nucleic acid molecule that is complementary thereto. In this regard, nucleic acid molecules at least 90%, preferably at least 95%, more preferably at least 98% or 99% identical over their entire length to the same are particularly preferred. Preferred embodiments in this respect are nucleic acid molecules that encode polypeptides which retain substantially the same biological function or activity as the INSP005 polypeptides.

The invention also provides a process for detecting a nucleic acid molecule of the invention, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting any such duplexes that are formed.

As discussed additionally below in connection with assays that may be utilised according to the invention, a nucleic acid molecule as described above may be used as a hybridization probe for RNA, cDNA or genomic DNA, in order to isolate full-length cDNAs and genomic clones encoding the INSP005 polypeptides and to isolate cDNA and genomic clones of homologous or orthologous genes that have a high sequence similarity to the gene encoding these polypeptides.

In this regard, the following techniques, among others known in the art, may be utilised and are discussed below for purposes of illustration. Methods for DNA sequencing and analysis are well known and are generally available in the art and may, indeed, be used to practice many of the embodiments of the invention discussed herein. Such methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proof-reading exonucleases such as those found in the ELONGASE Amplification System marketed by Gibco/BRL (Gaithersburg, MD). Preferably, the sequencing process may be automated using machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), the Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

One method for isolating a nucleic acid molecule encoding a polypeptide with an equivalent function to that of the INSP005 polypeptides is to probe a genomic or cDNA library with a natural or artificially-designed probe using standard procedures that are recognised in the art (see, for example, "Current Protocols in Molecular Biology", Ausubel

et al. (eds). Greene Publishing Association and John Wiley Interscience, New York, 1989,1992). Probes comprising at least 15, preferably at least 30, and more preferably at least 50, contiguous bases that correspond to, or are complementary to, nucleic acid sequences from the appropriate encoding gene (SEQ ID NO:13, SEQ ID NO:33 or SEQ ID NO:35), are particularly useful probes. Such probes may be labelled with an analytically-detectable reagent to facilitate their identification. Useful reagents include, but are not limited to, radioisotopes, fluorescent dyes and enzymes that are capable of catalysing the formation of a detectable product. Using these probes, the ordinarily skilled artisan will be capable of isolating complementary copies of genomic DNA, cDNA or RNA polynucleotides encoding proteins of interest from human, mammalian or other animal sources and screening such sources for related sequences, for example, for additional members of the family, type and/or subtype.

In many cases, isolated cDNA sequences will be incomplete, in that the region encoding the polypeptide will be cut short, normally at the 5' end. Several methods are available to obtain full length cDNAs, or to extend short cDNAs. Such sequences may be extended utilising a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed is based on the method of Rapid Amplification of cDNA Ends (RACE; see, for example, Frohman *et al.*, PNAS USA 85, 8998-9002, 1988). Recent modifications of this technique, exemplified by the MarathonTM technology (Clontech Laboratories Inc.), for example, have significantly simplified the search for longer cDNAs. A slightly different technique, termed "restriction-site" PCR, uses universal primers to retrieve unknown nucleic acid sequence adjacent a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). Inverse PCR may also be used to amplify or to extend sequences using divergent primers based on a known region (Triglia, T. *et al.* (1988) Nucleic Acids Res. 16:8186). Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. *et al.* (1991) PCR Methods Applic., 1, 111-119). Another method which may be used to retrieve unknown sequences is that of Parker, J.D. *et al.* (1991); Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinderTM libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences that contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

In one embodiment of the invention, the nucleic acid molecules of the present invention may be used for chromosome localisation. In this technique, a nucleic acid molecule is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important step in the confirmatory correlation of those sequences with the gene-associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular genomic region, any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleic acid molecule may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

The nucleic acid molecules of the present invention are also valuable for tissue localisation. Such techniques allow the determination of expression patterns of the polypeptide in tissues by detection of the mRNAs that encode them. These techniques include *in situ* hybridization techniques and nucleotide amplification techniques, such as PCR. Results from these studies provide an indication of the normal functions of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by a mutant gene provide valuable insights into the role of mutant polypeptides in disease. Such inappropriate expression may be of a temporal, spatial or quantitative nature.

Gene silencing approaches may also be undertaken to down-regulate endogenous expression of a gene encoding a polypeptide of the invention. RNA interference (RNAi) (Elbashir, SM *et al.*, Nature 2001, 411, 494-498) is one method of sequence specific post-transcriptional gene silencing that may be employed. Short dsRNA oligonucleotides are synthesised in vitro and introduced into a cell. The sequence specific binding of these dsRNA oligonucleotides triggers the degradation of target mRNA, reducing or ablating target protein expression.

Efficacy of the gene silencing approaches assessed above may be assessed through the measurement of polypeptide expression (for example, by Western blotting), and at the RNA level using TaqMan-based methodologies.

The vectors of the present invention comprise nucleic acid molecules of the invention and may be cloning or expression vectors. The host cells of the invention, which may be transformed, transfected or transduced with the vectors of the invention may be prokaryotic or eukaryotic.

The polypeptides of the invention may be prepared in recombinant form by expression of their encoding nucleic acid molecules in vectors contained within a host cell. Such expression methods are well known to those of skill in the art and many are described in detail by Sambrook *et al* (*supra*) and Fernandez & Hoeffler (1998, eds. "Gene expression systems. Using nature for the art of expression". Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto).

Generally, any system or vector that is suitable to maintain, propagate or express nucleic acid molecules to produce a polypeptide in the required host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those described in Sambrook *et al.*, (*supra*). Generally, the encoding gene can be placed under the control of a control element such as a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator, so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the transformed host cell.

Examples of suitable expression systems include, for example, chromosomal, episomal and virus-derived systems, including, for example, vectors derived from: bacterial plasmids, bacteriophage, transposons, yeast episomes, insertion elements, yeast chromosomal elements, viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses,

adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, or combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, including cosmids and phagemids. Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid.

Particularly suitable expression systems include microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (for example, baculovirus); plant cell systems transformed with virus expression vectors (for example, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (for example, Ti or pBR322 plasmids); or animal cell systems. Cell-free translation systems can also be employed to produce the polypeptides of the invention.

Introduction of nucleic acid molecules encoding a polypeptide of the present invention into host cells can be effected by methods described in many standard laboratory manuals, such as Davis *et al.*, Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, (*supra*). Particularly suitable methods include calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection (see Sambrook *et al.*, 1989 [*supra*]; Ausubel *et al.*, 1991 [*supra*]; Spector, Goldman & Leinwald, 1998). In eukaryotic cells, expression systems may either be transient (for example, episomal) or permanent (chromosomal integration) according to the needs of the system.

The encoding nucleic acid molecule may or may not include a sequence encoding a control sequence, such as a signal peptide or leader sequence, as desired, for example, for secretion of the translated polypeptide into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals. Leader sequences can be removed by the bacterial host in post-translational processing.

In addition to control sequences, it may be desirable to add regulatory sequences that allow for regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory sequences are those which cause the expression of a gene to be increased or decreased in response to a chemical or physical stimulus, including the

presence of a regulatory compound or to various temperature or metabolic conditions. Regulatory sequences are those non-translated regions of the vector, such as enhancers, promoters and 5' and 3' untranslated regions. These interact with host cellular proteins to carry out transcription and translation. Such regulatory sequences may vary in their strength and specificity. Depending on the vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript phagemid (Stratagene, LaJolla, CA) or pSportl™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (for example, heat shock, RUBISCO and storage protein genes) or from plant viruses (for example, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

An expression vector is constructed so that the particular nucleic acid coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the regulatory sequences being such that the coding sequence is transcribed under the "control" of the regulatory sequences, i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence. In some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame.

The control sequences and other regulatory sequences may be ligated to the nucleic acid coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector that already contains the control sequences and an appropriate restriction site.

For long-term, high-yield production of a recombinant polypeptide, stable expression is preferred. For example, cell lines which stably express the polypeptide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate

vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalised cell lines available from the American Type Culture Collection (ATCC) including, but not limited to, Chinese hamster ovary (CHO), HeLa, baby hamster kidney (BHK), monkey kidney (COS), C127, 3T3, BHK, HEK 293, Bowes melanoma and human hepatocellular carcinoma (for example Hep G2) cells and a number of other cell lines.

In the baculovirus system, the materials for baculovirus/insect cell expression systems are commercially available in kit form from, inter alia, Invitrogen, San Diego CA (the "MaxBac" kit). These techniques are generally known to those skilled in the art and are described fully in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Particularly suitable host cells for use in this system include insect cells such as Drosophila S2 and Spodoptera Sf9 cells.

There are many plant cell culture and whole plant genetic expression systems known in the art. Examples of suitable plant cellular genetic expression systems include those described in US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, Phytochemistry 30, 3861-3863 (1991).

In particular, all plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be utilised, so that whole plants are recovered which contain the transferred gene. Practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugar cane, sugar beet, cotton, fruit and other trees, legumes and vegetables.

Examples of particularly preferred bacterial host cells include *streptococci*, *staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells.

Examples of particularly suitable host cells for fungal expression include yeast cells (for

example, *S. cerevisiae*) and *Aspergillus* cells.

Any number of selection systems are known in the art that may be used to recover transformed cell lines. Examples include the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1980) Cell 22:817-23) genes that can be employed in tk- or aprt± cells, respectively.

Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dihydrofolate reductase (DHFR) that confers resistance to methotrexate (Wigler, M. *et al.* (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al* (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. Additional selectable genes have been described, examples of which will be clear to those of skill in the art.

Although the presence or absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the relevant sequence is inserted within a marker gene sequence, transformed cells containing the appropriate sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a polypeptide of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells that contain a nucleic acid sequence encoding a polypeptide of the invention and which express said polypeptide may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassays, for example, fluorescence activated cell sorting (FACS) or immunoassay techniques (such as the enzyme-linked immunosorbent assay [ELISA] and radioimmunoassay [RIA]), that include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein (see Hampton, R. *et al.* (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. *et al.* (1983) J. Exp. Med, 158, 1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridization or PCR probes for detecting sequences related to nucleic acid

molecules encoding polypeptides of the present invention include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled polynucleotide. Alternatively, the sequences encoding the polypeptide of the invention may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH)).

Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes and fluorescent, chemiluminescent or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Nucleic acid molecules according to the present invention may also be used to create transgenic animals, particularly rodent animals. Such transgenic animals form a further aspect of the present invention. This may be done locally by modification of somatic cells, or by germ line therapy to incorporate heritable modifications. Such transgenic animals may be particularly useful in the generation of animal models for drug molecules effective as modulators of the polypeptides of the present invention.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography is particularly useful for purification. Well known techniques for refolding proteins may be employed to regenerate an active conformation when the polypeptide is denatured during isolation and or purification.

Specialised vector constructions may also be used to facilitate purification of proteins, as desired, by joining sequences encoding the polypeptides of the invention to a nucleotide sequence encoding a polypeptide domain that will facilitate purification of soluble proteins. Examples of such purification-facilitating domains include metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilised metals, protein A domains that allow purification on immobilised immunoglobulin, and the

domain utilised in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the polypeptide of the invention may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing the polypeptide of the invention fused to several histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilised metal ion affinity chromatography as described in Porath, J. *et al.* (1992), Prot. Exp. Purif. 3: 263-281) while the thioredoxin or enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. *et al.* (1993; DNA Cell Biol. 12:441-453).

If the polypeptide is to be expressed for use in screening assays, generally it is preferred that it be produced at the surface of the host cell in which it is expressed. In this event, the host cells may be harvested prior to use in the screening assay, for example using techniques such as fluorescence activated cell sorting (FACS) or immunoaffinity techniques. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the expressed polypeptide. If polypeptide is produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

The polypeptide of the invention can be used to screen libraries of compounds in any of a variety of drug screening techniques. Such compounds may activate (agonise) or inhibit (antagonise) the level of expression of the gene or the activity of the polypeptide of the invention and form a further aspect of the present invention. Preferred compounds are effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

Agonist or antagonist compounds may be isolated from, for example, cells, cell-free preparations, chemical libraries or natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors or structural or functional mimetics. For a suitable review of such screening techniques, see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991).

Compounds that are most likely to be good antagonists are molecules that bind to the polypeptide of the invention without inducing the biological effects of the polypeptide

upon binding to it. Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to the polypeptide of the invention and thereby inhibit or extinguish its activity. In this fashion, binding of the polypeptide to normal cellular binding molecules may be inhibited, such that the normal biological activity of the polypeptide is prevented.

The polypeptide of the invention that is employed in such a screening technique may be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. In general, such screening procedures may involve using appropriate cells or cell membranes that express the polypeptide that are contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The functional response of the cells contacted with the test compound is then compared with control cells that were not contacted with the test compound. Such an assay may assess whether the test compound results in a signal generated by activation of the polypeptide, using an appropriate detection system. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist in the presence of the test compound is observed.

The INSP005 polypeptides of the present invention may modulate a variety of physiological and pathological processes, including reproductive processes such as egg maturation or fertilisation. Thus, the biological activity of the INSP005 polypeptides can be examined in systems that allow the study of such modulatory activities, using a variety of suitable assays. For example, possible assays include the measurement of oocyte fertilisation and/or pregnancy rates after ovulation induction, the measurement of embryo implantation rates, or in the case of male infertility the measurement of sperm motility (Luo C.W. *et al*, J. Biol. Chem. 276 (10), 6913-6921 (2001)).

A preferred method for identifying an agonist or antagonist compound of a polypeptide of the present invention comprises:

(a) contacting a cell expressing on the surface thereof the polypeptide according to the first aspect of the invention, the polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

(b) determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the polypeptide.

A further preferred method for identifying an agonist or antagonist of a polypeptide of the invention comprises:

(a) contacting a cell expressing on the surface thereof the polypeptide, the polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

(b) determining whether the compound binds to and activates or inhibits the polypeptide by comparing the level of a signal generated from the interaction of the compound with the polypeptide with the level of a signal in the absence of the compound.

In further preferred embodiments, the general methods that are described above may further comprise conducting the identification of agonist or antagonist in the presence of labelled or unlabelled ligand for the polypeptide.

In another embodiment of the method for identifying agonist or antagonist of a polypeptide of the present invention comprises:

determining the inhibition of binding of a ligand to cells which have a polypeptide of the invention on the surface thereof, or to cell membranes containing such a polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide, and determining the amount of ligand bound to the polypeptide. A compound capable of causing reduction of binding of a ligand is considered to be an agonist or antagonist. Preferably the ligand is labelled.

More particularly, a method of screening for a polypeptide antagonist or agonist compound comprises the steps of:

(a) incubating a labelled ligand with a whole cell expressing a polypeptide according to the invention on the cell surface, or a cell membrane containing a polypeptide of the invention,

(b) measuring the amount of labelled ligand bound to the whole cell or the cell membrane;

(c) adding a candidate compound to a mixture of labelled ligand and the whole cell or the cell membrane of step (a) and allowing the mixture to attain equilibrium;

(d) measuring the amount of labelled ligand bound to the whole cell or the cell membrane after step (c); and

(e) comparing the difference in the labelled ligand bound in step (b) and (d), such that the compound which causes the reduction in binding in step (d) is considered to be an agonist or antagonist.

The polypeptides may be found to modulate a variety of physiological and pathological processes in a dose-dependent manner in the above-described assays. Thus, the "functional equivalents" of the polypeptides of the invention include polypeptides that exhibit any of the same modulatory activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the polypeptides of the invention, preferably the "functional equivalents" will exhibit substantially similar dose-dependence in a given activity assay compared to the polypeptides of the invention.

In certain of the embodiments described above, simple binding assays may be used, in which the adherence of a test compound to a surface bearing the polypeptide is detected by means of a label directly or indirectly associated with the test compound or in an assay involving competition with a labelled competitor. In another embodiment, competitive drug screening assays may be used, in which neutralising antibodies that are capable of binding the polypeptide specifically compete with a test compound for binding. In this manner, the antibodies can be used to detect the presence of any test compound that possesses specific binding affinity for the polypeptide.

Assays may also be designed to detect the effect of added test compounds on the production of mRNA encoding the polypeptide in cells. For example, an ELISA may be constructed that measures secreted or cell-associated levels of polypeptide using monoclonal or polyclonal antibodies by standard methods known in the art, and this can be used to search for compounds that may inhibit or enhance the production of the

polypeptide from suitably manipulated cells or tissues. The formation of binding complexes between the polypeptide and the compound being tested may then be measured.

Assay methods that are also included within the terms of the present invention are those that involve the use of the genes and polypeptides of the invention in overexpression or ablation assays. Such assays involve the manipulation of levels of these genes/polypeptides in cells and assessment of the impact of this manipulation event on the physiology of the manipulated cells. For example, such experiments reveal details of signaling and metabolic pathways in which the particular genes/polypeptides are implicated, generate information regarding the identities of polypeptides with which the studied polypeptides interact and provide clues as to methods by which related genes and proteins are regulated.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the polypeptide of interest (see International patent application WO84/03564). In this method, large numbers of different small test compounds are synthesised on a solid substrate, which may then be reacted with the polypeptide of the invention and washed. One way of immobilising the polypeptide is to use non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques.

The polypeptide of the invention may be used to identify membrane-bound or soluble receptors, through standard receptor binding techniques that are known in the art, such as ligand binding and crosslinking assays in which the polypeptide is labelled with a radioactive isotope, is chemically modified, or is fused to a peptide sequence that facilitates its detection or purification, and incubated with a source of the putative receptor (for example, a composition of cells, cell membranes, cell supernatants, tissue extracts, or bodily fluids). The efficacy of binding may be measured using biophysical techniques such as surface plasmon resonance and spectroscopy. Binding assays may be used for the purification and cloning of the receptor, but may also identify agonists and antagonists of the polypeptide, that compete with the binding of the polypeptide to its receptor. Standard methods for conducting screening assays are well understood in the art.

The invention also includes a screening kit useful in the methods for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, that are described above.

The invention includes the agonists, antagonists, ligands, receptors, substrates and

enzymes, and other compounds which modulate the activity or antigenicity of the polypeptide of the invention discovered by the methods that are described above.

The invention also provides pharmaceutical compositions comprising a polypeptide, nucleic acid, ligand or compound of the invention in combination with a suitable pharmaceutical carrier. These compositions may be suitable as therapeutic or diagnostic reagents, as vaccines, or as other immunogenic compositions, as outlined in detail below.

According to the terminology used herein, a composition containing a polypeptide, nucleic acid, ligand or compound [X] is "substantially free of" impurities [herein, Y] when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95%, 98% or even 99% by weight.

The pharmaceutical compositions should preferably comprise a therapeutically effective amount of the polypeptide, nucleic acid molecule, ligand, or compound of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate, or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, for example, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise effective amount for a human subject will depend upon the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.05 mg/kg to 10 mg/kg. Compositions may be administered individually to a patient or may be administered in combination with other agents, drugs or hormones.

A pharmaceutical composition may also contain a pharmaceutically acceptable carrier, for administration of a therapeutic agent. Such carriers include antibodies and other polypeptides, genes and other therapeutic agents such as liposomes, provided that the

carrier does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulphates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

The pharmaceutical compositions utilised in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal or transcutaneous applications (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal means. Gene guns or hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

If the activity of the polypeptide of the invention is in excess in a particular disease state, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as described above, along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the polypeptide, such as by blocking the binding of ligands, substrates, enzymes, receptors, or by inhibiting a second signal, and thereby alleviating the abnormal condition. Preferably, such antagonists are antibodies. Most preferably, such antibodies are chimeric and/or humanised to minimise their immunogenicity, as described previously.

In another approach, soluble forms of the polypeptide that retain binding affinity for the ligand, substrate, enzyme, receptor, in question, may be administered. Typically, the polypeptide may be administered in the form of fragments that retain the relevant portions.

In an alternative approach, expression of the gene encoding the polypeptide can be inhibited using expression blocking techniques, such as the use of antisense nucleic acid molecules (as described above), either internally generated or separately administered. Modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions (signal sequence, promoters, enhancers and introns) of the gene encoding the polypeptide. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. *et al.* (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Such oligonucleotides may be administered or may be generated in situ from expression *in vivo*.

In addition, expression of the polypeptide of the invention may be prevented by using ribozymes specific to its encoding mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, *et al.*, Curr. Opin. Struct. Biol (1996) 6(4), 527-33). Synthetic ribozymes can be designed to specifically cleave mRNAs at selected positions thereby preventing translation of the mRNAs into functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate

backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be synthesised with non-natural backbones, for example, 2'-O-methyl RNA, to provide protection from ribonuclease degradation and may contain modified bases.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine and butosine, as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine and uridine which are not as easily recognised by endogenous endonucleases.

For treating abnormal conditions related to an under-expression of the polypeptide of the invention and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound that activates the polypeptide, i.e., an agonist as described above, to alleviate the abnormal condition. Alternatively, a therapeutic amount of the polypeptide in combination with a suitable pharmaceutical carrier may be administered to restore the relevant physiological balance of polypeptide.

Gene therapy may be employed to effect the endogenous production of the polypeptide by the relevant cells in the subject. Gene therapy is used to treat permanently the inappropriate production of the polypeptide by replacing a defective gene with a corrected therapeutic gene.

Gene therapy of the present invention can occur *in vivo* or *ex vivo*. *Ex vivo* gene therapy requires the isolation and purification of patient cells, the introduction of a therapeutic gene and introduction of the genetically altered cells back into the patient. In contrast, *in vivo* gene therapy does not require isolation and purification of a patient's cells.

The therapeutic gene is typically "packaged" for administration to a patient. Gene delivery vehicles may be non-viral, such as liposomes, or replication-deficient viruses, such as adenovirus as described by Berkner, K.L., in Curr. Top. Microbiol. Immunol., 158, 39-66 (1992) or adeno-associated virus (AAV) vectors as described by Muzyczka, N., in Curr. Top. Microbiol. Immunol., 158, 97-129 (1992) and U.S. Patent No. 5,252,479. For

example, a nucleic acid molecule encoding a polypeptide of the invention may be engineered for expression in a replication-defective retroviral vector. This expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding the polypeptide, such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo* (see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics (1996), T Strachan and A P Read, BIOS Scientific Publishers Ltd).

Another approach is the administration of "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue.

In situations in which the polypeptides or nucleic acid molecules of the invention are disease-causing agents, the invention provides that they can be used in vaccines to raise antibodies against the disease causing agent. Where the aforementioned polypeptide or nucleic acid molecule is one that is up-regulated, vaccine development can involve the raising of antibodies or T cells against such agents (as described in WO00/29428).

Vaccines according to the invention may either be prophylactic (ie. to prevent infection) or therapeutic (ie. to treat disease after infection). Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with pharmaceutically-acceptable carriers as described above, which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, H. pylori, and other pathogens.

Since polypeptides may be broken down in the stomach, vaccines comprising polypeptides are preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents.

The vaccine formulations of the invention may be presented in unit-dose or multi-dose containers. For example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Genetic delivery of antibodies that bind to polypeptides according to the invention may also be effected, for example, as described in International patent application WO98/55607.

The technology referred to as jet injection (see, for example, www.powderject.com) may also be useful in the formulation of vaccine compositions.

A number of suitable methods for vaccination and vaccine delivery systems are described in International patent application WO00/29428.

This invention also relates to the use of nucleic acid molecules according to the present invention as diagnostic reagents. Detection of a mutated form of the gene characterised by the nucleic acid molecules of the invention which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

Nucleic acid molecules for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR, ligase chain reaction (LCR), strand displacement amplification (SDA), or other amplification techniques (see Saiki *et al.*, Nature, 324, 163-166 (1986); Bej, *et al.*, Crit. Rev. Biochem. Molec. Biol., 26, 301-334 (1991); Birkenmeyer *et al.*, J. Virol. Meth., 35, 117-126 (1991); Van Brunt, J., Bio/Technology, 8, 291-294 (1990)) prior to analysis.

In one embodiment, this aspect of the invention provides a method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to the invention and comparing said level of expression to a control level, wherein a level that is different to said control level is indicative of disease. The method may comprise the steps of:

a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule of the invention and the probe;

b) contacting a control sample with said probe under the same conditions used in step a);

c) and detecting the presence of hybrid complexes in said samples;

wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of disease.

A further aspect of the invention comprises a diagnostic method comprising the steps of:

a) obtaining a tissue sample from a patient being tested for disease;

b) isolating a nucleic acid molecule according to the invention from said tissue sample; and

c) diagnosing the patient for disease by detecting the presence of a mutation in the nucleic acid molecule which is associated with disease.

To aid the detection of nucleic acid molecules in the above-described methods, an amplification step, for example using PCR, may be included.

Deletions and insertions can be detected by a change in the size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labelled RNA of the invention or alternatively, labelled antisense DNA sequences of the invention. Perfectly-matched sequences can be distinguished from mismatched duplexes by RNase digestion or by assessing differences in melting temperatures. The presence or absence of the mutation in the patient may be detected by contacting DNA with a nucleic acid probe that hybridises to the DNA under stringent conditions to form a hybrid double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation in the corresponding portion of the DNA strand.

Such diagnostics are particularly useful for prenatal and even neonatal testing.

Point mutations and other sequence differences between the reference gene and "mutant" genes can be identified by other well-known techniques, such as direct DNA sequencing or single-strand conformational polymorphism, (see Orita *et al.*, Genomics, 5, 874-879

(1989)). For example, a sequencing primer may be used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabelled nucleotides or by automatic sequencing procedures with fluorescent-tags. Cloned DNA segments may also be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. Further, point mutations and other sequence variations, such as polymorphisms, can be detected as described above, for example, through the use of allele-specific oligonucleotides for PCR amplification of sequences that differ by single nucleotides.

DNA sequence differences may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (for example, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc. Natl. Acad. Sci. USA (1985) 85: 4397-4401).

In addition to conventional gel electrophoresis and DNA sequencing, mutations such as microdeletions, aneuploidies, translocations, inversions, can also be detected by in situ analysis (see, for example, Keller *et al.*, DNA Probes, 2nd Ed., Stockton Press, New York, N.Y., USA (1993)), that is, DNA or RNA sequences in cells can be analysed for mutations without need for their isolation and/or immobilisation onto a membrane. Fluorescence in situ hybridization (FISH) is presently the most commonly applied method and numerous reviews of FISH have appeared (see, for example, Trachuck *et al.*, Science, 250, 559-562 (1990), and Trask *et al.*, Trends, Genet., 7, 149-154 (1991)).

In another embodiment of the invention, an array of oligonucleotide probes comprising a nucleic acid molecule according to the invention can be constructed to conduct efficient screening of genetic variants, mutations and polymorphisms. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee *et al.*, Science (1996), Vol 274, pp 610-613).

In one embodiment, the array is prepared and used according to the methods described in PCT application WO95/11995 (Chee *et al*); Lockhart, D. J. *et al.* (1996) Nat. Biotech: 14: 1675-1680); and Schena, M. *et al.* (1996) Proc. Natl. Acad. Sci. 93: 10614-10619).

Oligonucleotide pairs may range from two to over one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support. In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application WO95/25116 (Baldeschweiler *et al*). In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot blot apparatus), materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and over one million which lends itself to the efficient use of commercially-available instrumentation.

In addition to the methods discussed above, diseases may be diagnosed by methods comprising determining, from a sample derived from a subject, an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

Assay techniques that can be used to determine levels of a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art and are discussed in some detail above (including radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays). This aspect of the invention provides a diagnostic method which comprises the steps of: (a) contacting a ligand as described above with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

Protocols such as ELISA, RIA, and FACS for measuring polypeptide levels may additionally provide a basis for diagnosing altered or abnormal levels of polypeptide expression. Normal or standard values for polypeptide expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably

humans, with antibody to the polypeptide under conditions suitable for complex formation The amount of standard complex formation may be quantified by various methods, such as by photometric means.

Antibodies which specifically bind to a polypeptide of the invention may be used for the diagnosis of conditions or diseases characterised by expression of the polypeptide, or in assays to monitor patients being treated with the polypeptides, nucleic acid molecules, ligands and other compounds of the invention. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for the polypeptide include methods that utilise the antibody and a label to detect the polypeptide in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules known in the art may be used, several of which are described above.

Quantities of polypeptide expressed in subject, control and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease. Diagnostic assays may be used to distinguish between absence, presence, and excess expression of polypeptide and to monitor regulation of polypeptide levels during therapeutic intervention. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

A diagnostic kit of the present invention may comprise:

(a) a nucleic acid molecule of the present invention;

(b) a polypeptide of the present invention; or

(c) a ligand of the present invention.

In one aspect of the invention, a diagnostic kit may comprise a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to the invention; a second container containing primers useful for amplifying the nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease. The kit may further comprise a third container holding an agent for digesting unhybridised RNA.

In an alternative aspect of the invention, a diagnostic kit may comprise an array of nucleic

acid molecules, at least one of which may be a nucleic acid molecule according to the invention.

To detect polypeptide according to the invention, a diagnostic kit may comprise one or more antibodies that bind to a polypeptide according to the invention; and a reagent useful for the detection of a binding reaction between the antibody and the polypeptide.

Such kits will be of use in diagnosing a disease or susceptibility to diseases in which metalloproteases are implicated, particularly respiratory disorders, including emphysema and cystic fibrosis, metabolic disorders, cardiovascular disorders, bacterial infections, hypertension, proliferative disorders, including cancer, autoimmune/inflammatory disorders, including rheumatoid arthritis, neurological disorders, developmental disorders and reproductive disorders.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to INSP005 polypeptides.

It will be appreciated that modification of detail may be made without departing from the scope of the invention.

Brief description of the Figures

Figure 1: Summary of results of database searches using the INSP005 predicted polypeptide sequence as a query sequence (sequence alignments shown).

Figure 2: Table of human cDNA libraries used in the INSP005 cloning investigation.

Figure 3: Nucleotide sequence of the INSP005 predicted polypeptide and predicted amino acid sequence.

Figure 4: Table of INSP005 cloning primers.

Figure 5: 3'nucleotide and amino acid sequence of INSP005 identified by RACE PCR.

Figure 6: Table of primers used during INSP005 sequencing.

Figure 7: Putative full-length INSP005a cloned from human uterus cDNA.

Figure 8: INSP005a blastp vs. NCBI-nr database (top ten hits and top related alignment shown).

Figure 9: Map of PCR4-TOPO-IPAAAIPAAA7883-1 INSP005a cloning plasmid.

Figure 10: Putative full-length INSP005b cloned from a pool of cDNAs derived from human primary lung fibroblasts, keratinocytes and osteoarthritis synovium.

Figure 11: INSP005b blastp vs. NCBI-nr database (top ten hits and top related alignment shown).

Figure 12: Map of PCR-TOPO-IPAAA78836-2 INSP005b cloning plasmid.

Figure 13: Multiple alignment of the INSP005 predicted polypeptide sequence, the INSP005a cloned polypeptide sequence, the INSP005b cloned polypeptide sequence and certain prior art sequences of interest.

Figure 14: SignalP signal peptide prediction data for the INSP005b polypeptide

Examples

Example 1: INSP005 Predicted Polypeptide

An INSP005 polypeptide sequence (SEQ ID NO:37) predicted by proprietary bioinformatics techniques was used as a query sequence for searches of the following databases:

NCBI-nr	NCBI-nt	NCBI-pat-aa
NCBI-pat-nt	NCBI-month-aa	NCBI-month-nt
NCBI-est		

The results of these searches are summarised in Figure 1, which shows two relevant sequence alignments. The headings in Figure 1 indicate which searching/alignment algorithms were used and which database was searched. These results show that the closest related match to the INSP005 predicted polypeptide sequence is the hatching enzyme EHE4 from *Anguilla japonica* (Japanese eel). These searches also identified three other prior art sequences of interest, which are discussed in more detail below.

Members of the choriolysin/astacin-like family of metalloproteases have been implicated in chorion hardening of oviparous fish eggs after fertilisation (for an example see Shibata *et al.* (2000) J.Biol.Chem vol.275, No.12 p8349). This post-fertilisation change prevents

polyspermy and corresponds to the formation of fertilisation membranes in sea urchin, amphibian and the zona reaction in mammals. They have also been implicated in the hydrolysis of the hardened chorion at the time of hatching and the hydrolysis of unfertilised egg chorions.

As described above, the identification of novel metalloproteases is of extreme importance in increasing understanding of the underlying pathways that lead to certain disease states in which these proteins are implicated, and in developing more effective gene or drug therapies to treat these disorders. Similarly, the identification of further members of the astacin/choriolysin-like family of metalloproteases is of extreme importance in increasing understanding of the underlying pathways that lead to certain disease states in which these proteins are implicated, and in developing more effective gene or drug therapies to treat these disorders.

Example 2: Summary of INSP005 Cloning

1.1 cDNA libraries

Human cDNA libraries (in bacteriophage lambda (λ) vectors) were purchased from Stratagene or Clontech or prepared at the Serono Pharmaceutical Research Institute in λ ZAP or λ GT10 vectors according to the manufacturer's protocol (Stratagene). Bacteriophage λ DNA was prepared from small-scale cultures of infected *E.coli* host strain using the Wizard Lambda Preps DNA purification system according to the manufacturer's instructions (Promega, Corporation, Madison WI.). The list of libraries and host strains used is shown in Figure 2. Eight pools (A-H) of five different libraries (100 ng/μl phage DNA) were used in subsequent PCR reactions.

1.2 Generation of reverse transcribed cDNA templates

Total RNA was isolated from primary human cells, human cell lines and human tissues using the Trizol™ reagent (Invitrogen) according to the manufacturer's instructions or purchased from Clontech, Invitrogen or Ambion. The quality and concentration of the RNA was analysed using an Agilent 2100 Bioanalyzer.

For cDNA synthesis the reaction mixture contained: 1 μl oligo $(dT)_{15}$ primer (500 μg/ml, Promega cat. no. C 1101), 2 μg total RNA, 1 μl 10 mM dNTPs in a volume of 12 μl. The

mixture was heated to 65°C for 5 min and then chilled on ice. The following reagents were then added: 4 µl 5X first strand buffer, 2 µl DTT (0.1M), 1 µl RNAseOut recombinant ribonuclease inhibitor (40 units/µl, Promega, cat. no. N 2511) and incubated at 42°C for 2 min before addition of 1 µl (200 units) of Superscript II (Invitrogen cat. no. 18064-014). The mixture was incubated at 42°C for 50 min and then heated at 70°C for 15 min. To remove the RNA template, 1 µl (2 units) of *E. coli* RNase H (Invitrogen cat. no.18021-014) was added and the reaction mixture further incubated at 37°C for 20 min. The final reaction mix was diluted to 200 µl with sterile water and stored at –80°C. cDNA pools were generated by mixing equal volumes of 5 different cDNA templates.

1.3 PCR of virtual cDNAs from phage library DNA

A partial cDNA encoding INSP005 was obtained as a PCR amplification product of 248 bp using gene specific cloning primers (CP1 and CP2, Figure 3 and Figure 4). The PCR was performed in a final volume of 50 µl containing 1X AmpliTaq™ buffer, 200 µM dNTPs, 50 pmoles each of cloning primers primers, 2.5 units of AmpliTaq™ (Perkin Elmer) and 100 ng of each phage library pool DNA using an MJ Research DNA Engine, programmed as follows: 94°C, 1 min; 40 cycles of 94°C, 1 min, x °C, and y min and 72°C, (where x is the lowest Tm – 5°C and y = 1 min per kb of product); followed by 1 cycle at 72°C for 7 min and a holding cycle at 4°C.

The amplification products were visualized on 0.8 % agarose gels in 1 X TAE buffer (Invitrogen) and PCR products migrating at the predicted molecular mass were purified from the gel using the Wizard PCR Preps DNA Purification System (Promega). PCR products eluted in 50 µl of sterile water were either subcloned directly or stored at –20 °C.

1.4 Gene specific cloning primers for PCR

Pairs of PCR primers having a length of between 18 and 25 bases were designed for amplifying the full length and partial sequence of the virtual cDNA using Primer Designer Software (Scientific & Educational Software, PO Box 72045, Durham, NC 27722-2045, USA). PCR primers were optimized to have a Tm close to 55 ± 10 °C and a GC content of 40-60%. Primers were selected which had high selectivity for the target sequence INSP005 (little or no non-specific priming).

1.5 Subcloning of PCR Products

PCR products were subcloned into the topoisomerase I modified cloning vector (pCRII TOPO) using the TA cloning kit purchased from the Invitrogen Corporation using the conditions specified by the manufacturer. Briefly, 4 µl of gel purified PCR product from the human library pool N amplification was incubated for 15 min at room temperature with 1 µl of TOPO vector and 1 µl salt solution. The reaction mixture was then transformed into *E. coli* strain TOP10 (Invitrogen) as follows: a 50 µl aliquot of One Shot TOP10 cells was thawed on ice and 2 µl of TOPO reaction was added. The mixture was incubated for 15 min on ice and then heat shocked by incubation at 42°C for exactly 30s. Samples were returned to ice and 250µl of warm SOC media (room temperature) was added. Samples were incubated with shaking (220 rpm) for 1 h at 37°C. The transformation mixture was then plated on L-broth (LB) plates containing ampicillin (100 µg/ml) and incubated overnight at 37°C. Ampicillin resistant colonies containing cDNA inserts were identified by colony PCR.

1.6 Colony PCR

Colonies were inoculated into 50 µl sterile water using a sterile toothpick. A 10 µl aliquot of the inoculum was then subjected to PCR in a total reaction volume of 20 µl as described above, except the primers used were SP6 and T7. The cycling conditions were as follows: 94°C, 2 min; 30 cycles of 94°C, 30 sec, 47°C, 30 sec and 72°C for 1 min); 1 cycle, 72°C, 7 min. Samples were then maintained at 4°C (holding cycle) before further analysis.

PCR reaction products were analyzed on 1 % agarose gels in 1 X TAE buffer. Colonies which gave the expected PCR product size (248 bp cDNA + 185 bp due to the multiple cloning site or MCS) were grown up overnight at 37°C in 5 ml L-Broth (LB) containing ampicillin (100 µg /ml), with shaking at 220 rpm at 37°C.

1.7 Plasmid DNA preparation and Sequencing

Miniprep plasmid DNA was prepared from 5 ml cultures using a Qiaprep Turbo 9600 robotic system (Qiagen) or Wizard Plus SV Minipreps kit (Promega cat. no. 1460) according to the manufacturer's instructions. Plasmid DNA was eluted in 100 µl of sterile water. The DNA concentration was measured using an Eppendorf BO photometer. Plasmid

DNA (200-500 ng) was subjected to DNA sequencing with T7 primer and SP6 primer using the BigDyeTerminator system (Applied Biosystems cat. no. 4390246) according to the manufacturer's instructions. Sequencing reactions were purified using Dye-Ex columns (Qiagen) or Montage SEQ 96 cleanup plates (Millipore cat. no. LSKS09624) then analyzed on an Applied Biosystems 3700 sequencer.

1.8 Identification of the full length sequence of INSP005 using RACE PCR.

The predicted sequence of the INSP005 ORF is shown in Figure 3. Attempts to isolate the full length coding sequence by PCR failed on the libraries tested, using primer pairs to amplify the full length prediction or a shorter version which uses a 2nd predicted start site at M96 in the open reading frame. The closest related sequences to INSP005 are the astacin-like metallopeptidase in *Anguilla japonica* and choriolysin H in *Oryzias latipes*. INSP005 appears to be a human orthologue of choriolysin H. Choriolysins are implicated in chorion hardening of oviparous fish eggs after fertilization, suggesting that uterus may be a suitable source of the INSP005 mRNA. The choice of this tissue was further supported by the finding of a single EST, BI061462 derived from a human uterus tumour.

In order to identify the full coding sequence, RACE PCR was performed on cDNA prepared from uterus RNA (purchased from Clontech) using the GeneRacer kit (Invitrogen cat no. L1502-01) according to the manufacturer's instructions. For amplification of 3' ends, the first PCR was performed in a 50 µl reaction volume containing 1 µl RACE Ready cDNA, 5 µl of 10X High Fidelity buffer,1 µl of dNTPs (10 mM), 2 µl of 50 mM $MgSO_4$, 3 µl of GeneRacer 3' primer (10 µM), 1 µl of gene specific primer (78836-GR1-3') (10 µM) and 2.5 units (0.5 µl) of Platinum Taq DNA polymerase Hi Fi (Invitrogen). The cycling conditions were as follows: 94°C, 2 min; 5 cycles of 94°C 30 s and 72°C 2min; 5 cycles of 94°C, 30 s and 70°C, 5 min; 25 cycles of 94°C, 30 s, 65°C 30 s and 68°C 5 min; a final extension at 68°C for 10 min and a holding cycle of 4°C. One µl of the amplification reaction was then used as a template for a nested PCR which was performed in a final reaction volume of 50 µl with the same reagents as above except for the primers. The primers for the nested PCR were 1 µl of GeneRacer 3' nested primer (10 µM) and 1 µl of nested gene specific primer (78836-GR1nest-3') (10 µM). The cycling conditions were 94°C, 2 min; 25 cycles of 94°C, 30 s, 65°C, 30 s and 68°C, 5 min; a final extension at 68°C for 10 min and a holding cycle of 4°C. PCR products were gel purified, subcloned into

pCR4-TOPO vector and sequenced as described above. All primers used are listed in Figure 4. The nucleotide sequence and amino acid sequence of the 3' RACE product is shown in Figure 5. The amino acid sequence encoded by the 3' RACE product has an extended C-terminal, diverging from the prediction after nucleotide position 85 which was suggestive of an alternatively spliced form.

1.9 Cloning of the full length coding sequence of INSP005 by PCR

The putative full length coding sequence of INSP005 was cloned from human uterus cDNA (prepared as described in section 1.2) by PCR in a 50 µl PCR reaction mixture as containing 2 µl uterus cDNA, 5 µl of 10X High Fidelity buffer,1 µl of dNTPs (10 mM), 2 µl of 50 mM $MgSO_4$, 1 µl of gene specific primer 78836-FL-F (10 µM), 1 µl of reverse gene specific primer 78836-FL-R (10 µM) and 2.5 units (0.5 µl) of Platinum Taq DNA polymerase Hi Fi (Invitrogen). The cycling conditions were 94^0C, 2 min; 40 cycles of 94^0C, 30 s, 55^0C, 30 s and 68^0C, 1 min 30 s min; a final extension at 68^0C for 10 min and a holding cycle of 4^0C. The amplification products were visualized on 0.8 % agarose gels in 1 X TAE buffer (Invitrogen) and PCR products migrating at the predicted molecular mass (1048 bp) were purified from the gel using the Wizard PCR Preps DNA Purification System (Promega). PCR products were eluted in 50 µl of sterile water and subcloned into pCR4 TOPO vector as described in section 1.4. Several ampicilin resistant colonies were subjected to colony PCR as described in section 1.5 except that the extension time in the amplification reaction was 2 min. Colonies containing the correct size insert (1048 bp + 99 bp due to the MCS) were grown up overnight at 37 ^{0}C in 5 ml L-Broth (LB) containing ampicillin (100 µg /ml), with shaking at 220 rpm at 37 ^{0}C. Miniprep plasmid DNA was prepared from 5 ml cultures using a Qiaprep Turbo 9600 robotic system (Qiagen) or Wizard Plus SV Minipreps kit (Promega cat. no. 1460) according to the manufacturer's instructions and 200-500 ng of mini-prep DNA was sequenced as described in section 1.7 with T3 and T7 primers (Figure 6). The cloned sequence is given in Figure 7. The amino acid alignment of the cloned sequence (INSP005a) with the predicted sequence is shown in Figure 13. The map of the resultant plasmid, pCR4-TOPO-IPAAA78836-1 (SEQ ID NO:38; plasmid ID. No. 13164) is shown in Figure 9.

2.0 Identification of cDNA libraries/templates containing INSP005

PCR products obtained with CP1 and CP2 and migrating at the correct size (248 bp) were identified in library pool N (libraries 18, 19, 20 and 21). A cDNA encoding a putative full length INSP005 (INSP005a) was isolated from uterus cDNA using 78836-FL-F and 78836-FL-R primers. Primer 78836-FL-F is located in exon 3 of the predicted sequence. No PCR products were obtained using the reverse primer (78836-FL-R) with primers located in exon 1 of the prediction.

A second putative full length version of INSP005 (INSP005b) containing an alternative 5' end was cloned from a pool of cDNAs derived from human primary lung fibroblasts, keratinocytes and osteoarthritis synovium using primers 78836-FL2-F and 78836-FL-R but was not detected in uterus. The resultant PCR product (1313 bp – Figure 10) was subcloned into pCR4 TOPO vector using the TOPO-TA cloning kit and sequenced as described in sections 1.5 -1.7. The map of the resultant plasmid, pCR4-TOPO-IPAAA78836-2 (SEQ ID NO:39; plasmid ID. No. 13296) is shown in Figure 12.

2.1 Summary of Cloning Results

Attempts to clone the full-length INSP005 predicted polypeptide identified two variants of the INSP005 predicted polypeptide, herein referred to as INSP005a and INSP005b (Figure 13; SEQ ID NO:14 and SEQ ID NO:34, respectively). As described above, the INSP005a and INSP005b polypeptides (and the INSP005b mature polypeptide) are herein referred to as the INSP005 polypeptides, as distinct from the INSP005 predicted polypeptide.

The nucleotide and amino acid sequences for the predicted exons within the INSP005a and INSP005b polypeptides are given in SEQ ID NOs 1-12 and SEQ ID NOs 15-32, respectively. As described above, the putative full-length nucleotide sequences of the INSP005a and INSP005b polypeptides are given in SEQ ID NOs 13 and 33, respectively. The amino acid sequences of the INSP005a and INSP005b polypeptides are given in SEQ ID NOs 14 and 34, respectively.

The relationships between the INSP005a and INSP005b polypeptides and the INSP005 predicted polypeptide and three prior art sequences of interest are shown in Figure 13, which provides a sequence-level alignment of the sequences. These relationships will now be described in detail.

INSP005a is a putative full-length version of the INSP005 predicted polypeptide from a uterus cDNA library. This sequence differs from the original INSP005 prediction in that it has a truncated 5' end, starting at methionine 3 of the original INSP005 predicted polypeptide (see Figure 13). INSP005a also has an extended 3' end that incorporates an extra exon relative to the INSP005 predicted polypeptide. INSP005a has six predicted exons in total. These differences were not predicted due to the low homology of those sequence elements to other metalloproteinases. In addition, there is an alternative amino acid used at position 22 of INSP005a compared to the INSP005 predicted polypeptide. INSP005a is not predicted to contain a signal peptide. INSP005a has no in frame alternative upstream start methionine before an upstream STOP codon.

The polypeptide sequence shown in SEQ ID NO:14 (INSP005a), was used as a BLAST query against the NCBI non-redundant sequence database. The top ten hits are all egg hatching-related enzymes from *Anguilla japonica* or choriolytic proteases and align to the query sequence with highly significant E-values (from e^{-115} to $2e^{-41}$) (Figure 8). Figure 8 also shows the alignment of the INSP005a polypeptide query sequence to the sequence of the top biochemically annotated hit, the hatching enzyme HE13 from *Anguilla japonica.* These results provide strong evidence that the INSP005a polypeptide is a metalloprotease, more specifically that it is a choriolysin/astacin-like metalloprotease.

INSP005b is a putative full-length version of the INSP005 predicted polypeptide cloned from a pool of cDNAs derived from human primary lung fibroblasts, keratinocytes and osteoarthritis synovium. INSP005b subsumes the original INSP005 predicted polypeptide sequence, though two alternative amino acids are used at positions 117 and 222. It also contains three new upstream exons and one downstream exon, making INSP005b a nine exon polypeptide. The final exon is shared with INSP005a. INSP005b was not detected in uterus. These differences were not predicted due to the low homology of those sequence elements to other metalloproteinases. As described above, INSP005b is predicted to contain a signal peptide with a cleavage site between amino acids 23 and 24 (SEQ ID NOs 35 and 36; Figure 14).

The polypeptide sequence shown in SEQ ID NO:34 (INSP005b), was used as a BLAST query against the NCBI non-redundant sequence database. The top ten hits are all egg hatching-related enzymes from *Anguilla japonica* or choriolytic proteases and align to the

query sequence with highly significant E-values (from e^{-152} to $4e^{-46}$) (Figure 11). Figure 11 also shows the alignment of the cloned polypeptide query sequence to the sequence of the top biochemically annotated hit, the hatching enzyme HE13 from *Anguilla japonica*. These results provide strong evidence that the INSP005b polypeptide is a metalloprotease, more specifically that it is a choriolysin/astacin-like metalloprotease.

The first 7 exons of INSP005b match a nucleotide sequence disclosed in WO200216566-A2, given accession number AX443328 (see Figure 1 and Figure 13), although the final 3' exon is not disclosed in WO200216566-A2 (Applera Corp). The nucleotide and polypeptide molecules of the present invention specifically exclude those disclosed in WO200216566-A2.

A further prior art sequence of interest is a spliced EST (BI061462.1; see Figure 1) from uterus tumour covering exon 1 of INSP005a and exons 2, 3 and 4 of INSP005b. The direction of the EST is not given in the report and it is hard to come to a conclusion about the presence of a start methionine from the translation. However, the nucleotide and polypeptide molecules of the present invention specifically exclude the sequences disclosed in EST BI061462.1.

Another prior art sequence of interest, with accession number AX526191 (Lexicon) (Figures 1 and 13), is described as cDNA in the relevant database entry (disclosed in WO02/066624) and no reference is made to a possible reproductive role. It subsumes INSP005a and exons 2-8 of INSP005b. However, an alternative amino acid is used at position 127 in INSP005a compared to the corresponding amino acid in INSP005b and the AX526191 (Lexicon) sequence. The start methionine of AX526191 is covered by the uterus tumour EST described above. A signal peptide is predicted for AX526191with a probability of 0.875. The nucleotide and polypeptide molecules of the present invention do not include the sequences disclosed in WO02/066624, including that with accession number AX526191.

Figure 13 also highlights the active site residues, which are identical in each of the polypeptides shown. This provides further compelling evidence that the INSP005a and INSP005b polypeptides are metalloproteases.

The INSP005a and INSP005b polypeptides therefore represent novel metalloproteases, and there is strong evidence that they are members of the choriolysin/astacin-like family of metalloproteases. The INSP005a and INSP005b polypeptides may therefore play important roles in physiological and pathological processes in humans, particularly in reproductive processes.

Sequence Listing:

SEQ ID NO: 1 (INSP005A nucleotide sequence exon 1)

```
  1 ATGGGTGGTA GTGGTGTCGT GGAGGTCCCC TTCCTGCTCT CCAGCAAGTA
 51 CG
```

SEQ ID NO: 2 (INSP005A protein sequence exon 1)

```
  1 MGGSGVVEVP FLLSSKYD
```

SEQ ID NO: 3 (INSP005A nucleotide sequence exon 2)

```
  1 ATGAGCCCAG CCGCCAGGTC ATCCTGGAGG CTCTTGCGGA GTTTGAACGT
 51 TCCACGTGCA TCAGGTTTGT CACCTATCAG GACCAGAGAG ACTTCATTTC
101 CATCATCCCC ATGTATGG
```

SEQ ID NO: 4 (INSP005A protein sequence exon 2)

```
  1 EPSRQVILEA LAEFERSTCI RFVTYQDQRD FISIIPMYG
```

SEQ ID NO: 5 (INSP005A nucleotide sequence exon 3)

```
  1 GTGCTTCTCG AGTGTGGGGC GCAGTGGAGG GATGCAGGTG GTCTCCCTGG
 51 CGCCCACGTG TCTCCAGAAG GGCCGGGGCA TTGTCCTTCA TGAGCTCATG
101 CATGTGCTGG GCTTCTGGCA CGAGCACACG CGGGCCGACC GGGACCGCTA
151 TATCCGTGTC AACTGGAACG AGATCCTGCC AG
```

SEQ ID NO: 6 (INSP005A protein sequence exon 3)

```
  1 CFSSVGRSGG MQVVSLAPTC LQKGRGIVLH ELMHVLGFWH EHTRADRDRY
 51 IRVNWNEILP G
```

SEQ ID NO: 7 (INSP005A nucleotide sequence exon 4)

```
  1 GCTTTGAAAT CAACTTCATC AAGTCTCAGA GCAGCAACAT GCTGACGCCC
 51 TATGACTACT CCTCTGTGAT GCACTATGGG AG
```

SEQ ID NO: 8 (INSP005A protein sequence exon 4)

```
  1 FEINFIKSQS SNMLTPYDYS SVMHYGR
```

SEQ ID NO: 9 (INSP005A nucleotide sequence exon 5)

```
  1 GCTCGCCTTC AGCCGGCGTG GGCTGCCCAC CATCACACCA CTTTGGGCCC
 51 CCAGTGTCCA CATCGGCCAG CGATGGAACC TGAGTGCCTC GGACATCACC
```

101 CGGGTCCTCA AACTCTACGG CTGCAGCCCA AGTGGCCCCA GGCCCCGTGG

151 GAGAG

SEQ ID NO: 10 (INSP005A protein sequence exon 5)

1 LAFSRRGLPT ITPLWAPSVH IGQRWNLSAS DITRVLKLYG CSPSGPRPRG

51 RG

SEQ ID NO: 11 (INSP005A nucleotide sequence exon 6)

1 GGTCCCATGC CCACAGCACT GGTAGGAGCC CCGCCCCGGC CTCCCTATCT

51 CTGCAGCGGC TTTTGGAGGC ACTGTCGGCG GAATCCAGGA GCCCCGACCC

101 CAGTGGTTCC AGTGCGGGAG GCCAGCCCGT TCCTGCAGGG CCTGGGGAGA

151 GCCCACATGG GTGGGAGTCC CCTGCCCTGA AAAAGCTCAG TGCAGAGGCC

201 TCGGCAAGGC AGCCTCAGAC CCTAGCTTCC TCCCCAAGAT CAAGGCCTGG

251 AGCAGGTGCC CCCGGTGTTG CTCAGGAGCA GTCCTGGCTG GCCGGAGTGT

301 CCACCAAGCC CACAGTCCCA TCTTCAGAAG CAGGAATCCA GCCAGTCCCT

351 GTCCAGGGAA GCCCAGCTCT GCCAGGGGGC TGTGTACCTA GAAATCATTT

401 CAAGGGGATG TCCGAAGAT

SEQ ID NO: 12 (INSP005A protein sequence exon 6)

1 SHAHSTGRSP APASLSLQRL LEALSAESRS PDPSGSSAGG QPVPAGPGES

51 PHGWESPALK KLSAEASARQ PQTLASSPRS RPGAGAPGVA QEQSWLAGVS

101 TKPTVPSSEA GIQPVPVQGS PALPGGCVPR NHFKGMSED

SEQ ID NO: 13 (INSP005A full nucleotide sequence)

1 ATGGGTGGTA GTGGTGTCGT GGAGGTCCCC TTCCTGCTCT CCAGCAAGTA

51 CGATGAGCCC AGCCGCCAGG TCATCCTGGA GGCTCTTGCG GAGTTTGAAC

101 GTTCCACGTG CATCAGGTTT GTCACCTATC AGGACCAGAG AGACTTCATT

151 TCCATCATCC CCATGTATGG GTGCTTCTCG AGTGTGGGGC GCAGTGGAGG

201 GATGCAGGTG GTCTCCCTGG CGCCCACGTG TCTCCAGAAG GGCCGGGGCA

251 TTGTCCTTCA TGAGCTCATG CATGTGCTGG GCTTCTGGCA CGAGCACACG

301 CGGGCCGACC GGGACCGCTA TATCCGTGTC AACTGGAACG AGATCCTGCC

351 AGGCTTTGAA ATCAACTTCA TCAAGTCTCA GAGCAGCAAC ATGCTGACGC

401 CCTATGACTA CTCCTCTGTG ATGCACTATG GGAGGCTCGC CTTCAGCCGG

451 CGTGGGCTGC CCACCATCAC ACCACTTTGG GCCCCCAGTG TCCACATCGG

501 CCAGCGATGG AACCTGAGTG CCTCGGACAT CACCCGGGTC CTCAAACTCT

551 ACGGCTGCAG CCCAAGTGGC CCCAGGCCCC GTGGGAGAGG GTCCCATGCC

601 CACAGCACTG GTAGGAGCCC CGCCCCGGCC TCCCTATCTC TGCAGCGGCT

651 TTTGGAGGCA CTGTCGGCGG AATCCAGGAG CCCCGACCCC AGTGGTTCCA

701 GTGCGGGAGG CCAGCCCGTT CCTGCAGGGC CTGGGGAGAG CCCACATGGG

751 TGGGAGTCCC CTGCCCTGAA AAAGCTCAGT GCAGAGGCCT CGGCAAGGCA

801 GCCTCAGACC CTAGCTTCCT CCCCAAGATC AAGGCCTGGA GCAGGTGCCC

851 CCGGTGTTGC TCAGGAGCAG TCCTGGCTGG CCGGAGTGTC CACCAAGCCC

901 ACAGTCCCAT CTTCAGAAGC AGGAATCCAG CCAGTCCCTG TCCAGGGAAG

951 CCCAGCTCTG CCAGGGGGCT GTGTACCTAG AAATCATTTC AAGGGGATGT

1001 CCGAAGAT

SEQ ID NO: 14 (INSP005A full protein sequence)

1 MGGSGVVEVP FLLSSKYDEP SRQVILEALA EFERSTCIRF VTYQDQRDFI

51 SIIPMYGCFS SVGRSGGMQV VSLAPTCLQK GRGIVLHELM HVLGFWHEHT

101 RADRDRYIRV NWNEILPGFE INFIKSQSSN MLTPYDYSSV MHYGRLAFSR

151 RGLPTITPLW APSVHIGQRW NLSASDITRV LKLYGCSPSG PRPRGRGSHA

201 HSTGRSPAPA SLSLQRLLEA LSAESRSPDP SGSSAGGQPV PAGPGESPHG

251 WESPALKKLS AEASARQPQT LASSPRSRPG AGAPGVAQEQ SWLAGVSTKP

301 TVPSSEAGIQ PVPVQGSPAL PGGCVPRNHF KGMSED

SEQ ID NO: 15 (INSP005B nucleotide sequence exon 1)

1 ATGGAGGGTG TAGGGGGTCT CTGGCCTTGG GTGCTGGGTC TGCTCTCCTT

51 GCCAG

SEQ ID NO: 16 (INSP005B protein sequence exon 1)

1 MEGVGGLWPW VLGLLSLPG

SEQ ID NO: 17 (INSP005B nucleotide sequence exon 2)

1 GTGTGATCCT AGGAGCGCCC CTGGCCTCCA GCTGCGCAGG AGCCTGTGGT

51 ACCAGCTTCC CAGATGGCCT CACCCCTGAG GGAACCCAGG CCTCCGGGGA

101 CAAGGACATT CCTGCAATTA ACCAAG

SEQ ID NO: 18 (INSP005B protein sequence exon 2)

1 VILGAPLASS CAGACGTSFP DGLTPEGTQA SGDKDIPAIN QG

SEQ ID NO: 19 (INSP005B nucleotide sequence exon 3)

1 GGCTCATCCT GGAAGAAACC CCAGAGAGCA GCTTCCTCAT CGAGGGGGAC

51 ATCATCCGGC CG

SEQ ID NO: 20 (INSP005B protein sequence exon 3)

1 LILEETPESS FLIEGDIIRP

SEQ ID NO: 21 (INSP005B nucleotide sequence exon 4)

1 AGTCCCTTCC GACTGCTGTC AGCAACCAGC AACAAATGGC CCATGGGTGG

51 TAGTGGTGTC GTGGAGGTCC CCTTCCTGCT CTCCAGCAAG TACG

SEQ ID NO: 22 (INSP005B protein sequence exon 4)

1 SPFRLLSATS NKWPMGGSGV VEVPFLLSSK YD

SEQ ID NO: 23 (INSP005B nucleotide sequence exon 5)

1 ATGAGCCCAG CCGCCAGGTC ATCCTGGAGG CTCTTGCGGA GTTTGAACGT

51 TCCACGTGCA TCAGGTTTGT CACCTATCAG GACCAGAGAG ACTTCATTTC

101 CATCATCCCC ATGTATGG

SEQ ID NO: 24 (INSP005B protein sequence exon 5)

1 EPSRQVILEA LAEFERSTCI RFVTYQDQRD FISIIPMYG

SEQ ID NO: 25 (INSP005B nucleotide sequence exon 6)

1 GTGCTTCTCG AGTGTGGGGC GCAGTGGAGG GATGCAGGTG GTCTCCCTGG

51 CGCCCACGTG TCTCCAGAAG GGCCGGGGCA TTGTCCTTCA TGAGCTCATG

101 CATGTGCTGG GCTTCTGGCA CGAGCACACG CGGGCCGACC GGGACCGCTA

151 TATCCGTGTC AACTGGAACG AGATCCTGCC AG

SEQ ID NO: 26 (INSP005B protein sequence exon 6)

1 CFSSVGRSGG MQVVSLAPTC LQKGRGIVLH ELMHVLGFWH EHTRADRDRY

51 IRVNWNEILP G

SEQ ID NO: 27 (INSP005B nucleotide sequence exon 7)

1 GCTTTGAAAT CAACTTCATC AAGTCTCGGA GCAGCAACAT GCTGACGCCC

51 TATGACTACT CCTCTGTGAT GCACTATGGG AG

SEQ ID NO: 28 (INSP005B protein sequence exon 7)

```
  1 FEINFIKSRS SNMLTPYDYS SVMHYGR
```

SEQ ID NO: 29 (INSP005B nucleotide sequence exon 8)

```
  1 GCTCGCCTTC AGCCGGCGTG GGCTGCCCAC CATCACACCA CTTTGGGCCC
 51 CCAGTGTCCA CATCGGCCAG CGATGGAACC TGAGTGCCTC GGACATCACC
101 CGGGTCCTCA AACTCTACGG CTGCAGCCCA AGTGGCCCCA GGCCCCGTGG
151 GAGAG
```

SEQ ID NO: 30 (INSP005B protein sequence exon 8)

```
  1 LAFSRRGLPT ITPLWAPSVH IGQRWNLSAS DITRVLKLYG CSPSGPRPRG
 51 RG
```

SEQ ID NO: 31 (INSP005B nucleotide sequence exon 9)

```
  1 GGTCCCATGC CCACAGCACT GGTAGGAGCC CCGCTCCGGC CTCCCTATCT
 51 CTGCAGCGGC TTTTGGAGGC ACTGTCGGCG GAATCCAGGA GCCCCGACCC
101 CAGTGGTTCC AGTGCGGGAG GCCAGCCCGT TCCTGCAGGG CCTGGGGAGA
151 GCCCACATGG GTGGGAGTCC CCTGCCCTGA AAAAGCTCAG TGCAGAGGCC
201 TCGGCAAGGC AGCCTCAGAC CCTAGCTTCC TCCCCAAGAT CAAGGCCTGG
251 AGCAGGTGCC CCCGGTGTTG CTCAGGAGCA GTCCTGGCTG GCCGGAGTGT
301 CCACCAAGCC CACAGTCCCA TCTTCAGAAG CAGGAATCCA GCCAGTCCCT
351 GTCCAGGGAA GCCCAGCTCT GCCAGGGGGC TGTGTACCTA GAAATCATTT
401 CAAGGGGATG TCCGAAGAT
```

SEQ ID NO: 32 (INSP005B protein sequence exon 9)

```
  1 SHAHSTGRSP APASLSLQRL LEALSAESRS PDPSGSSAGG QPVPAGPGES
 51 PHGWESPALK KLSAEASARQ PQTLASSPRS RPGAGAPGVA QEQSWLAGVS
101 TKPTVPSSEA GIQPVPVQGS PALPGGCVPR NHFKGMSED
```

SEQ ID NO: 33 (INSP005B full nucleotide sequence)

```
  1 ATGGAGGGTG TAGGGGGTCT CTGGCCTTGG GTGCTGGGTC TGCTCTCCTT
 51 GCCAGGTGTG ATCCTAGGAG CGCCCCTGGC CTCCAGCTGC GCAGGAGCCT
101 GTGGTACCAG CTTCCCAGAT GGCCTCACCC CTGAGGGAAC CCAGGCCTCC
151 GGGGACAAGG ACATTCCTGC AATTAACCAA GGGCTCATCC TGGAAGAAAC
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201 CCCAGAGAGC AGCTTCCTCA TCGAGGGGGA CATCATCCGG CCGAGTCCCT
251 TCCGACTGCT GTCAGCAACC AGCAACAAAT GGCCCATGGG TGGTAGTGGT
301 GTCGTGGAGG TCCCCTTCCT GCTCTCCAGC AAGTACGATG AGCCCAGCCG
351 CCAGGTCATC CTGGAGGCTC TTGCGGAGTT TGAACGTTCC ACGTGCATCA
401 GGTTTGTCAC CTATCAGGAC CAGAGAGACT TCATTTCCAT CATCCCCATG
451 TATGGGTGCT TCTCGAGTGT GGGGCGCAGT GGAGGGATGC AGGTGGTCTC
501 CCTGGCGCCC ACGTGTCTCC AGAAGGGCCG GGGCATTGTC CTTCATGAGC
551 TCATGCATGT GCTGGGCTTC TGGCACGAGC ACACGCGGGC CGACCGGGAC
601 CGCTATATCC GTGTCAACTG GAACGAGATC CTGCCAGGCT TTGAAATCAA
651 CTTCATCAAG TCTCGGAGCA GCAACATGCT GACGCCCTAT GACTACTCCT
701 CTGTGATGCA CTATGGGAGG CTCGCCTTCA GCCGGCGTGG GCTGCCCACC
751 ATCACACCAC TTTGGGCCCC CAGTGTCCAC ATCGGCCAGC GATGGAACCT
801 GAGTGCCTCG GACATCACCC GGGTCCTCAA ACTCTACGGC TGCAGCCCAA
851 GTGGCCCCAG GCCCCGTGGG AGAGGGTCCC ATGCCCACAG CACTGGTAGG
901 AGCCCCGCTC CGGCCTCCCT ATCTCTGCAG CGGCTTTTGG AGGCACTGTC
951 GGCGGAATCC AGGAGCCCCG ACCCCAGTGG TTCCAGTGCG GGAGGCCAGC
1001 CCGTTCCTGC AGGGCCTGGG GAGAGCCCAC ATGGGTGGGA GTCCCCTGCC
1051 CTGAAAAAGC TCAGTGCAGA GGCCTCGGCA AGGCAGCCTC AGACCCTAGC
1101 TTCCTCCCCA AGATCAAGGC CTGGAGCAGG TGCCCCCGGT GTTGCTCAGG
1151 AGCAGTCCTG GCTGGCCGGA GTGTCCACCA AGCCCACAGT CCCATCTTCA
1201 GAAGCAGGAA TCCAGCCAGT CCCTGTCCAG GGAAGCCCAG CTCTGCCAGG
1251 GGGCTGTGTA CCTAGAAATC ATTTCAAGGG GATGTCCGAA GAT
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SEQ ID NO: 34 (INSP005B full protein sequence)

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  1 MEGVGGLWPW VLGLLSLPGV ILGAPLASSC AGACGTSFPD GLTPEGTQAS
 51 GDKDIPAINQ GLILEETPES SFLIEGDIIR PSPFRLLSAT SNKWPMGGSG
101 VVEVPFLLSS KYDEPSRQVI LEALAEFERS TCIRFVTYQD QRDFISIIPM
151 YGCFSSVGRS GGMQVVSLAP TCLQKGRGIV LHELMHVLGF WHEHTRADRD
201 RYIRVNWNEI LPGFEINFIK SRSSNMLTPY DYSSVMHYGR LAFSRRGLPT
251 ITPLWAPSVH IGQRWNLSAS DITRVLKLYG CSPSGPRPRG RGSHAHSTGR
301 SPAPASLSLQ RLLEALSAES RSPDPSGSSA GGQPVPAGPG ESPHGWESPA
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351 LKKLSAEASA RQPQTLASSP RSRPGAGAPG VAQEQSWLAG VSTKPTVPSS

401 EAGIQPVPVQ GSPALPGGCV PRNHFKGMSE D

SEQ ID NO: 35 (INSP005 mature nucleotide sequence)

```
   1 GCGCCCCTGG CCTCCAGCTG CGCAGGAGCC TGTGGTACCA GCTTCCCAGA
  51 TGGCCTCACC CCTGAGGGAA CCCAGGCCTC CGGGGACAAG GACATTCCTG
 101 CAATTAACCA AGGGCTCATC CTGGAAGAAA CCCCAGAGAG CAGCTTCCTC
 151 ATCGAGGGGG ACATCATCCG GCCGAGTCCC TTCCGACTGC TGTCAGCAAC
 201 CAGCAACAAA TGGCCCATGG GTGGTAGTGG TGTCGTGGAG GTCCCCTTCC
 251 TGCTCTCCAG CAAGTACGAT GAGCCCAGCC GCCAGGTCAT CCTGGAGGCT
 301 CTTGCGGAGT TTGAACGTTC CACGTGCATC AGGTTTGTCA CCTATCAGGA
 351 CCAGAGAGAC TTCATTTCCA TCATCCCCAT GTATGGGTGC TTCTCGAGTG
 401 TGGGGCGCAG TGGAGGGATG CAGGTGGTCT CCCTGGCGCC CACGTGTCTC
 451 CAGAAGGGCC GGGGCATTGT CCTTCATGAG CTCATGCATG TGCTGGGCTT
 501 CTGGCACGAG CACACGCGGG CCGACCGGGA CCGCTATATC CGTGTCAACT
 551 GGAACGAGAT CCTGCCAGGC TTTGAAATCA ACTTCATCAA GTCTCGGAGC
 601 AGCAACATGC TGACGCCCTA TGACTACTCC TCTGTGATGC ACTATGGGAG
 651 GCTCGCCTTC AGCCGGCGTG GGCTGCCCAC CATCACACCA CTTTGGGCCC
 701 CCAGTGTCCA CATCGGCCAG CGATGGAACC TGAGTGCCTC GGACATCACC
 751 CGGGTCCTCA AACTCTACGG CTGCAGCCCA AGTGGCCCCA GGCCCCGTGG
 801 GAGAGGGTCC CATGCCCACA GCACTGGTAG GAGCCCCGCT CCGGCCTCCC
 851 TATCTCTGCA GCGGCTTTTG GAGGCACTGT CGGCGGAATC CAGGAGCCCC
 901 GACCCCAGTG GTTCCAGTGC GGGAGGCCAG CCCGTTCCTG CAGGGCCTGG
 951 GGAGAGCCCA CATGGGTGGG AGTCCCCTGC CCTGAAAAAG CTCAGTGCAG
1001 AGGCCTCGGC AAGGCAGCCT CAGACCCTAG CTTCCTCCCC AAGATCAAGG
1051 CCTGGAGCAG GTGCCCCCGG TGTTGCTCAG GAGCAGTCCT GGCTGGCCGG
1101 AGTGTCCACC AAGCCCACAG TCCCATCTTC AGAAGCAGGA ATCCAGCCAG
1151 TCCCTGTCCA GGGAAGCCCA GCTCTGCCAG GGGGCTGTGT ACCTAGAAAT
1201 CATTTCAAGG GGATGTCCGA AGAT
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SEQ ID NO: 36 (INSP005 mature polypeptide sequence)

```
   1 APLASSCAGA CGTSFPDGLT PEGTQASGDK DIPAINQGLI LEETPESSFL
  51 IEGDIIRPSP FRLLSATSNK WPMGGSGVVE VPFLLSSKYD EPSRQVILEA
 101 LAEFERSTCI RFVTYQDQRD FISIIPMYGC FSSVGRSGGM QVVSLAPTCL
 151 QKGRGIVLHE LMHVLGFWHE HTRADRDRYI RVNWNEILPG FEINFIKSRS
 201 SNMLTPYDYS SVMHYGRLAF SRRGLPTITP LWAPSVHIGQ RWNLSASDIT
 251 RVLKLYGCSP SGPRPRGRGS HAHSTGRSPA PASLSLQRLL EALSAESRSP
 301 DPSGSSAGGQ PVPAGPGESP HGWESPALKK LSAEASARQP QTLASSPRSR
 351 PGAGAPGVAQ EQSWLAGVST KPTVPSSEAG IQPVPVQGSP ALPGGCVPRN
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401 HFKGMSED

SEQ ID NO: 37 (INSP005 Predicted Polypeptide Sequence)

```
  1  MLRLWDFNPG GALSDLALGL RGMEEGGYSC AGACGTSFPD GLTPEGTQAS GDKDIPAINQ
 61  GLILEETPES SFLIEGDIIR PSPFRLLSAT SNKWPMGGSG VVEVPFLLSS KYDEPSHQVI
121  LEALAEFERS TCIRFVTYQD QRDFISIIPM YGCFSSVGRS GGMQVVSLAP TCLQKGRGIV
181  LHELMHVLGF WHEHTRADRD RYIRVNWNEI LPGFEINFIK SQSSNMLTPY DYSSVMHYGR
241  LAFSRRGLPT ITPLWAPSVH IGQRWNLSAS DITRVLKLYG CSPSGPRPRG RGEWHGRKVT
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SEQ ID NO: 38 (pCR4 TOPO IPAAA78836-1 plasmid nucleotide sequence)

```
   1  AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC
  61  ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC
 121  TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
 181  TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTCA
 241  GAATTAACCC TCACTAAAGG GACTAGTCCT GCAGGTTTAA ACGAATTCGC CCTTAGCCAC
 301  AGGCTTAATC TTCGGACATC CCCTTGAAAT GATTTCTAGG TACACAGCCC CCTGGCAGAG
 361  CTGGGCTTCC CTGGACAGGG ACTGGCTGGA TTCCTGCTTC TGAAGATGGG ACTGTGGGCT
 421  TGGTGGACAC TCCGGCCAGC CAGGACTGCT CCTGAGCAAC ACCGGGGGCA CCTGCTCCAG
 481  GCCTTGATCT TGGGGAGGAA GCTAGGGTCT GAGGCTGCCT TGCCGAGGCC TCTGCACTGA
 541  GCTTTTTCAG GGCAGGGGAC TCCCACCCAT GTGGGCTCTC CCCAGGCCCT GCAGGAACGG
 601  GCTGGCCTCC CGCACTGGAA CCACTGGGGT CGGGGCTCCT GGATTCCGCC GACAGTGCCT
 661  CCAAAAGCCG CTGCAGAGAT AGGGAGGCCG GGGCGGGGCT CCTACCAGTG CTGTGGGCAT
 721  GGGACCCTCT CCCACGGGGC CTGGGGCCAC TTGGGCTGCA GCCGTAGAGT TTGAGGACCC
 781  GGGTGATGTC CGAGGCACTC AGGTTCCATC GCTGGCCGAT GTGGACACTG GGGGCCCAAA
 841  GTGGTGTGAT GGTGGGCAGC CCACGCCGGC TGAAGGCGAG CCTCCCATAG TGCATCACAG
 901  AGGAGTAGTC ATAGGGCGTC AGCATGTTGC TGCTCTGAGA CTTGATGAAG TTGATTTCAA
 961  AGCCTGGCAG GATCTCGTTC CAGTTGACAC GGATATAGCG GTCCCGGTCG GCCCGCGTGT
1021  GCTCGTGCCA GAAGCCCAGC ACATGCATGA GCTCATGAAG GACAATGCCC CGGCCCTTCT
1081  GGAGACACGT GGGCGCCAGG GAGACCACCT GCATCCCTCC ACTGCGCCCC ACACTCGAGA
1141  AGCACCCATA CATGGGGATG ATGGAAATGA AGTCTCTCTG GTCCTGATAG GTGACAAACC
1201  TGATGCACGT GGAACGTTCA AACTCCGCAA GAGCCTCCAG GATGACCTGG CGGCTGGGCT
1261  CATCGTACTT GCTGGAGAGC AGGAAGGGGA CCTCCACGAC ACCACTACCA CCCATGGGCC
1321  ATTTGTTGCT GGTTGCTGAC AGAAGGGCGA ATTCGCGGCC GCTAAATTCA ATTCGCCCTA
1381  TAGTGAGTCG TATTACAATT CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC
1441  TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCT TTCGCCAGCT GGCGTAATAG
1501  CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTATACG TACGGCAGTT
1561  TAAGGTTTAC ACCTATAAAA GAGAGAGCCG TTATCGTCTG TTTGTGGATG TACAGAGTGA
1621  TATTATTGAC ACGCCGGGGC GACGGATGGT GATCCCCCTG GCCAGTGCAC GTCTGCTGTC
1681  AGATAAAGTC TCCCGTGAAC TTTACCCGGT GGTGCATATC GGGGATGAAA GCTGGCGCAT
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1741  GATGACCACC GATATGGCCA GTGTGCCGGT CTCCGTTATC GGGGAAGAAG TGGCTGATCT
1801  CAGCCACCGC GAAAATGACA TCAAAAACGC CATTAACCTG ATGTTCTGGG GAATATAAAT
1861  GTCAGGCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTCACGT AGAAAGCCAG
1921  TCCGCAGAAA CGGTGCTGAC CCCGGATGAA TGTCAGCTAC TGGGCTATCT GGACAAGGGA
1981  AAACGCAAGC GCAAAGAGAA AGCAGGTAGC TTGCAGTGGG CTTACATGGC GATAGCTAGA
2041  CTGGGCGGTT TTATGGACAG CAAGCGAACC GGAATTGCCA GCTGGGGCGC CCTCTGGTAA
2101  GGTTGGGAAG CCCTGCAAAG TAAACTGGAT GGCTTTCTCG CCGCCAAGGA TCTGATGGCG
2161  CAGGGGATCA AGCTCTGATC AAGAGACAGG ATGAGGATCG TTTCGCATGA TTGAACAAGA
2221  TGGATTGCAC GCAGGTTCTC CGGCCGCTTG GGTGGAGAGG CTATTCGGCT ATGACTGGGC
2281  ACAACAGACA ATCGGCTGCT CTGATGCCGC CGTGTTCCGG CTGTCAGCGC AGGGGCGCCC
2341  GGTTCTTTTT GTCAAGACCG ACCTGTCCGG TGCCCTGAAT GAACTGCAAG ACGAGGCAGC
2401  GCGGCTATCG TGGCTGGCCA CGACGGGCGT TCCTTGCGCA GCTGTGCTCG ACGTTGTCAC
2461  TGAAGCGGGA AGGGACTGGC TGCTATTGGG CGAAGTGCCG GGGCAGGATC TCCTGTCATC
2521  TCACCTTGCT CCTGCCGAGA AAGTATCCAT CATGGCTGAT GCAATGCGGC GGCTGCATAC
2581  GCTTGATCCG GCTACCTGCC CATTCGACCA CCAAGCGAAA CATCGCATCG AGCGAGCACG
2641  TACTCGGATG GAAGCCGGTC TTGTCGATCA GGATGATCTG GACGAAGAGC ATCAGGGGCT
2701  CGCGCCAGCC GAACTGTTCG CCAGGCTCAA GGCGAGCATG CCCGACGGCG AGGATCTCGT
2761  CGTGACCCAT GGCGATGCCT GCTTGCCGAA TATCATGGTG GAAAATGGCC GCTTTTCTGG
2821  ATTCATCGAC TGTGGCCGGC TGGGTGTGGC GGACCGCTAT CAGGACATAG CGTTGGCTAC
2881  CCGTGATATT GCTGAAGAGC TTGGCGGCGA ATGGGCTGAC CGCTTCCTCG TGCTTTACGG
2941  TATCGCCGCT CCCGATTCGC AGCGCATCGC CTTCTATCGC CTTCTTGACG AGTTCTTCTG
3001  AATTATTAAC GCTTACAATT TCCTGATGCG GTATTTTCTC CTTACGCATC TGTGCGGTAT
3061  TTCACACCGC ATACAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA
3121  TTTTTCTAAA TACATTCAAA TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT
3181  CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC
3241  TTTTTTGCGG CATTTTGCCT TCCTGTTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAAA
3301  GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTTACATCG AACTGGATCT CAACAGCGGT
3361  AAGATCCTTG AGAGTTTTCG CCCCGAAGAA CGTTTTCCAA TGATGAGCAC TTTTAAAGTT
3421  CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGGC AAGAGCAACT CGGTCGCCGC
3481  ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG
3541  GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA CCATGAGTGA TAACACTGCG
3601  GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC TAACCGCTTT TTTGCACAAC
3661  ATGGGGGATC ATGTAACTCG CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA
3721  AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA CAACGTTGCG CAAACTATTA
3781  ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT
3841  AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA
3901  TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG CACTGGGGCC AGATGGTAAG
3961  CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT
4021  AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT
4081  TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT AATTTAAAAG GATCTAGGTG
4141  AAGATCCTTT TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGA
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4201  GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA
4261  ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG TGGTTTGTTT GCCGGATCAA
4321  GAGCTACCAA CTCTTTTTCC GAAGGTAACT GGCTTCAGCA GAGCGCAGAT ACCAAATACT
4381  GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA
4441  TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT
4501  ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC AGCGGTCGGG CTGAACGGGG
4561  GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG
4621  CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA
4681  AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT
4741  CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC GTCGATTTTT GTGATGCTCG
4801  TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG CCTTTTTACG GTTCCTGGGC
4861  TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTC TGTGGATAAC
4921  CGTATTACCG CCTTTGAGTG AGCTGATACC GCTCGCCGCA GCCGAACGAC CGAGCGCAGC
4981  GAGTCAGTGA GCGAGGAAGC GGAAG
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SEQ ID NO: 39 (XpCR4TOPO IPAAA78836-2 plasmid nucleotide sequence)

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   1  AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC
  61  ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC
 121  TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
 181  TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GCCAAGCTCA
 241  GAATTAACCC TCACTAAAGG GACTAGTCCT GCAGGTTTAA ACGAATTCGC CCTTAGCCAC
 301  AGGCTTAATC TTCGGACATC CCCTTGAAAT GATTTCTAGG TACACAGCCC CCTGGCAGAG
 361  CTGGGCTTCC CTGGACAGGG ACTGGCTGGA TTCCTGCTTC TGAAGATGGG ACTGTGGGCT
 421  TGGTGGACAC TCCGGCCAGC CAGGACTGCT CCTGAGCAAC ACCGGGGGCA CCTGCTCCAG
 481  GCCTTGATCT TGGGGAGGAA GCTAGGGTCT GAGGCTGCCT TGCCGAGGCC TCTGCACTGA
 541  GCTTTTTCAG GGCAGGGGAC TCCCACCCAT GTGGGCTCTC CCCAGGCCCT GCAGGAACGG
 601  GCTGGCCTCC CGCACTGGAA CCACTGGGGT CGGGGCTCCT GGATTCCGCC GACAGTGCCT
 661  CCAAAAGCCG CTGCAGAGAT AGGGAGGCCG GAGCGGGGCT CCTACCAGTG CTGTGGGCAT
 721  GGGACCCTCT CCCACGGGGC CTGGGGCCAC TTGGGCTGCA GCCGTAGAGT TTGAGGACCC
 781  GGGTGATGTC CGAGGCACTC AGGTTCCATC GCTGGCCGAT GTGGACACTG GGGGCCCAAA
 841  GTGGTGTGAT GGTGGGCAGC CCACGCCGGC TGAAGGCGAG CCTCCCATAG TGCATCACAG
 901  AGGAGTAGTC ATAGGGCGTC AGCATGTTGC TGCTCCGAGA CTTGATGAAG TTGATTTCAA
 961  AGCCTGGCAG GATCTCGTTC CAGTTGACAC GGATATAGCG GTCCCGGTCG GCCCGCGTGT
1021  GCTCGTGCCA GAAGCCCAGC ACATGCATGA GCTCATGAAG GACAATGCCC CGGCCCTTCT
1081  GGAGACACGT GGGCGCCAGG GAGACCACCT GCATCCCTCC ACTGCGCCCC ACACTCGAGA
1141  AGCACCCATA CATGGGGATG ATGGAAATGA AGTCTCTCTG GTCCTGATAG GTGACAAACC
1201  TGATGCACGT GGAACGTTCA AACTCCGCAA GAGCCTCCAG GATGACCTGG CGGCTGGGCT
1261  CATCGTACTT GCTGGAGAGC AGGAAGGGGA CCTCCACGAC ACCACTACCA CCCATGGGCC
1321  ATTTGTTGCT GGTTGCTGAC AGCAGTCGGA AGGGACTCGG CCGGATGATG TCCCCCTCGA
1381  TGAGGAAGCT GCTCTCTGGG GTTTCTTCCA GGATGAGCCC TTGGTTAATT GCAGGAATGT
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1441  CCTTGTCCCC GGAGGCCTGG GTTCCCTCAG GGGTGAGGCC ATCTGGGAAG CTGGTACCAC
1501  AGGCTCCTGC GCAGCTGGAG GCCAGGGGCG CTCCTAGGAT CACACCTGGC AAGGAGAGCA
1561  GACCCAGCAC CCAAGGCCAG AGACCCCCTA CACCCTCCAT GGTAGAAAGG GCGAATTCGC
1621  GGCCGCTAAA TTCAATTCGC CCTATAGTGA GTCGTATTAC AATTCACTGG CCGTCGTTTT
1681  ACAACGTCGT GACTGGGAAA ACCCTGGCGT TACCCAACTT AATCGCCTTG CAGCACATCC
1741  CCCTTTCGCC AGCTGGCGTA ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT
1801  GCGCAGCCTA TACGTACGGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG
1861  TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCG GGGCGACGGA TGGTGATCCC
1921  CCTGGCCAGT GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA
1981  TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT
2041  TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA
2101  CCTGATGTTC TGGGGAATAT AAATGTCAGG CATGAGATTA TCAAAAAGGA TCTTCACCTA
2161  GATCCTTTTC ACGTAGAAAG CCAGTCCGCA GAAACGGTGC TGACCCCGGA TGAATGTCAG
2221  CTACTGGGCT ATCTGGACAA GGGAAAACGC AAGCGCAAAG AGAAAGCAGG TAGCTTGCAG
2281  TGGGCTTACA TGGCGATAGC TAGACTGGGC GGTTTTATGG ACAGCAAGCG AACCGGAATT
2341  GCCAGCTGGG GCGCCCTCTG GTAAGGTTGG GAAGCCCTGC AAAGTAAACT GGATGGCTTT
2401  CTCGCCGCCA AGGATCTGAT GGCGCAGGGG ATCAAGCTCT GATCAAGAGA CAGGATGAGG
2461  ATCGTTTCGC ATGATTGAAC AAGATGGATT GCACGCAGGT TCTCCGGCCG CTTGGGTGGA
2521  GAGGCTATTC GGCTATGACT GGGCACAACA GACAATCGGC TGCTCTGATG CCGCCGTGTT
2581  CCGGCTGTCA GCGCAGGGGC GCCCGGTTCT TTTTGTCAAG ACCGACCTGT CCGGTGCCCT
2641  GAATGAACTG CAAGACGAGG CAGCGCGGCT ATCGTGGCTG GCCACGACGG GCGTTCCTTG
2701  CGCAGCTGTG CTCGACGTTG TCACTGAAGC GGGAAGGGAC TGGCTGCTAT TGGGCGAAGT
2761  GCCGGGGCAG GATCTCCTGT CATCTCACCT TGCTCCTGCC GAGAAAGTAT CCATCATGGC
2821  TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC TGCCCATTCG ACCACCAAGC
2881  GAAACATCGC ATCGAGCGAG CACGTACTCG GATGGAAGCC GGTCTTGTCG ATCAGGATGA
2941  TCTGGACGAA GAGCATCAGG GGCTCGCGCC AGCCGAACTG TTCGCCAGGC TCAAGGCGAG
3001  CATGCCCGAC GGCGAGGATC TCGTCGTGAC CCATGGCGAT GCCTGCTTGC CGAATATCAT
3061  GGTGGAAAAT GGCCGCTTTT CTGGATTCAT CGACTGTGGC CGGCTGGGTG TGGCGGACCG
3121  CTATCAGGAC ATAGCGTTGG CTACCCGTGA TATTGCTGAA GAGCTTGGCG GCGAATGGGC
3181  TGACCGCTTC CTCGTGCTTT ACGGTATCGC CGCTCCCGAT TCGCAGCGCA TCGCCTTCTA
3241  TCGCCTTCTT GACGAGTTCT TCTGAATTAT TAACGCTTAC AATTTCCTGA TGCGGTATTT
3301  TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACAG GTGGCACTTT TCGGGGAAAT
3361  GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG
3421  AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA
3481  CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC
3541  CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC
3601  ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT
3661  CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC
3721  GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA
3781  CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC
3841  ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG
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3901  GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA
3961  CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG
4021  GCAACAACGT TGCGCAAACT ATTAACTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA
4081  TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG
4141  GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT
4201  GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT
4261  CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG
4321  CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT
4381  TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT
4441  TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT
4501  TGAGATCCTT TTTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAAACC ACCGCTACCA
4561  GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC
4621  AGCAGAGCGC AGATACCAAA TACTGTCCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC
4681  AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT
4741  GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG
4801  GCGCAGCGGT CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGGA GCGAACGACC
4861  TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA GCGCCACGCT TCCCGAAGGG
4921  AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG
4981  CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCG GGTTTCGCCA CCTCTGACTT
5041  GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC
5101  GCGGCCTTTT TACGGTTCCT GGGCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCCTGCG
5161  TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC
5221  CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAG
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CLAIMS

1. A polypeptide, which polypeptide is a secreted polypeptide and which:

i) comprises the amino acid sequence as recited in SEQ ID NO:14;

ii) is a fragment thereof which functions as a secreted protein of the metalloprotease class or has an antigenic determinant in common with the polypeptides of (i); or

iii) is a functional equivalent of (i) or (ii).

2. A polypeptide according to claim 1 which consists of the sequence recited in SEQ ID NO:14 or is a functional equivalent thereof.

3. A polypeptide, which polypeptide is a secreted polypeptide and which:

i) comprises the amino acid sequence as recited in SEQ ID NO:34 or SEQ ID NO:36;

ii) is a fragment thereof which functions as a secreted protein of the metalloprotease class or has an antigenic determinant in common with the polypeptides of (i); or

iii) is a functional equivalent of (i) or (ii).

4. A polypeptide according to claim 1 which consists of the sequence recited in SEQ ID NO:34 or SEQ ID NO:36 or is a functional equivalent thereof.

5. A polypeptide which is a functional equivalent according to any one of claims 1-4, which is homologous to the amino acid sequence as recited in SEQ ID NO:14, SEQ ID NO:34 or SEQ ID NO:36, and has metalloprotease activity.

6. A fragment or functional equivalent according to any one of the preceding claims, which has greater than 80% sequence identity with the amino acid sequence recited in SEQ ID NO:14, SEQ ID NO:34 or SEQ ID NO:36 or with active fragments thereof, preferably greater than 85%, 90%, 95%, 98% or 99% sequence identity.

7. A purified nucleic acid molecule which encodes a polypeptide according to any one of the preceding claims.

8. A purified nucleic acid molecule according to claim 7, which has the nucleic acid

sequence as recited in SEQ ID NO:13, SEQ ID NO:33 or SEQ ID NO:35 or is a redundant equivalent or fragment thereof.

9. A purified nucleic acid molecule which hydridizes under high stringency conditions with a nucleic acid molecule according to claim 7 or claim 8.

10. A vector comprising a nucleic acid molecule as recited in any one of claims 7-9.

11. The vector of claim 10 wherein said vector is the PCR-TOPO-IPAAA78836-1 vector (SEQ ID NO:38).

12. The vector of claim 10 wherein said vector is the PCR-TOPO-IPAAA78836-2 vector (SEQ ID NO:39).

13. A host cell transformed with a vector according to any one of claims 10-12.

14. A ligand which binds specifically to, and which preferably inhibits the metalloprotease activity of, a polypeptide according to any one of claims 1-6.

15. A ligand according to claim 14, which is an antibody.

16. A compound that either increases or decreases the level of expression or activity of a polypeptide according to any one of claims 1-6.

17. A compound according to claim 16 that binds to a polypeptide according to any one of claims 1-6 without inducing any of the biological effects of the polypeptide.

18. A compound according to claim 17, which is a natural or modified substrate, ligand, enzyme, receptor or structural or functional mimetic.

19. A polypeptide according to any one of claim 1-6, a nucleic acid molecule according to any one of claims 7-9, a vector according to any one of claims 10-12, a host cell according to claim 13, a ligand according to claim 14 or 15, or a compound according to any one of claims 16-18, for use in therapy or diagnosis of disease.

20. A method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to any one of claim 1-6, or assessing the activity of a polypeptide according to any one of claim 1-6, in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of disease.

21. A method according to claim 20 that is carried out *in vitro*.

22. A method according to claim 20 or claim 21, which comprises the steps of: (a) contacting a ligand according to claim 14 or claim 15 with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

23. A method according to claim 20 or claim 21, comprising the steps of:

a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule according to any one of claims 7-9 and the probe;

b) contacting a control sample with said probe under the same conditions used in step a); and

c) detecting the presence of hybrid complexes in said samples; wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of disease.

24. A method according to claim 20 or claim 21, comprising:

a) contacting a sample of nucleic acid from tissue of the patient with a nucleic acid primer under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule according to any one of claims 7-9 and the primer;

b) contacting a control sample with said primer under the same conditions used in step a); and

c) amplifying the sampled nucleic acid; and

d) detecting the level of amplified nucleic acid from both patient and control samples; wherein detection of levels of the amplified nucleic acid in the patient sample that differ significantly from levels of the amplified nucleic acid in the control sample is indicative of disease.

25. A method according to claim 20 or claim 21 comprising:

a) obtaining a tissue sample from a patient being tested for disease;

b) isolating a nucleic acid molecule according to any one of claims 7-9 from said tissue sample; and

c) diagnosing the patient for disease by detecting the presence of a mutation which is associated with disease in the nucleic acid molecule as an indication of the disease.

26. The method of claim 25, further comprising amplifying the nucleic acid molecule to form an amplified product and detecting the presence or absence of a mutation in the amplified product.

27. The method of either claim 25 or 26, wherein the presence or absence of the mutation in the patient is detected by contacting said nucleic acid molecule with a nucleic acid probe that hybridises to said nucleic acid molecule under stringent conditions to form a hybrid double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation.

28. A method according to any one of claims 20-27, wherein said disease is a respiratory disorder, including emphysema and cystic fibrosis, a metabolic disorder, a cardiovascular disorder, a bacterial infection, hypertension, a proliferative disorder, including cancer, an autoimmune/inflammatory disorder, including rheumatoid arthritis, a neurological disorder, a developmental disorder, a reproductive disorder or other pathological condition in which metalloproteases are implicated.

29. Use of a polypeptide according to any one of claims 1-6 as a secreted protein, preferably as a metalloprotease.

30. A pharmaceutical composition comprising polypeptide according to any one of claim 1-6, a nucleic acid molecule according to any one of claims 7-9, a vector according to any one of claims 10-12, a host cell according to claim 13, a ligand according to claim 14 or 15, or a compound according to any one of claims 16-18.

31. A vaccine composition comprising a polypeptide according to any one of claims 1-6 or a nucleic acid molecule according to any one of claims 7-9.

32. A polypeptide according to any one of claim 1-6, a nucleic acid molecule according to any one of claims 7-9, a vector according to any one of claims 10-12, a host cell according to claim 13, a ligand according to claim 14 or 15, or a compound

according to any one of claims 16-18, or a pharmaceutical composition according to claim 30, for use in the manufacture of a medicament for the treatment of a respiratory disorder, including emphysema and cystic fibrosis, a metabolic disorder, a cardiovascular disorder, a bacterial infection, hypertension, a proliferative disorder, including cancer, an autoimmune/inflammatory disorder, including rheumatoid arthritis, a neurological disorder, a developmental disorder, a reproductive disorder or other pathological condition in which metalloproteases are implicated.

33. A method of treating a disease in a patient, comprising administering to the patient polypeptide according to any one of claim 1-6, a nucleic acid molecule according to any one of claims 7-9, a vector according to any one of claims 10-12, a host cell according to claim 13, a ligand according to claim 14 or 15, or a compound according to any one of claims 16-18, or a pharmaceutical composition according to claim 30.

34. A method according to claim 33, wherein, for diseases in which the expression of the natural gene or the activity of the polypeptide is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, ligand, compound or composition administered to the patient is an agonist.

35. A method according to claim 33, wherein, for diseases in which the expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, ligand, compound or composition administered to the patient is an antagonist.

36. A method of monitoring the therapeutic treatment of disease in a patient, comprising monitoring over a period of time the level of expression or activity of a polypeptide according to any one of claims 1-6, or the level of expression of a nucleic acid molecule according to any one of claims 7-9 in tissue from said patient, wherein altering said level of expression or activity over the period of time towards a control level is indicative of regression of said disease.

37. A method for the identification of a compound that is effective in the treatment and/or diagnosis of disease, comprising contacting a polypeptide according to any

one of claims 1-6, or a nucleic acid molecule according to any one of claims 7-9 with one or more compounds suspected of possessing binding affinity for said polypeptide or nucleic acid molecule, and selecting a compound that binds specifically to said nucleic acid molecule or polypeptide.

38. A kit useful for diagnosing disease comprising a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to any one of claims 7-9; a second container containing primers useful for amplifying said nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease.

39. The kit of claim 38, further comprising a third container holding an agent for digesting unhybridised RNA.

40. A kit comprising an array of nucleic acid molecules, at least one of which is a nucleic acid molecule according to any one of claims 7-9.

41. A kit comprising one or more antibodies that bind to a polypeptide as recited in any one of claims 1-6; and a reagent useful for the detection of a binding reaction between said antibody and said polypeptide.

42. A transgenic or knockout non-human animal that has been transformed to express higher, lower or absent levels of a polypeptide according to any one of claims 1-6.

43. A method for screening for a compound effective to treat disease, by contacting a non-human transgenic animal according to claim 42 with a candidate compound and determining the effect of the compound on the disease of the animal.

Figure 1: Results of database searches using the INSP005 predicted polypeptide sequence as the query sequence

Blastp vs. NCBI-nr

```
>dbj|BAB68513.1| hatching enzyme EHE4 [Anguilla japonica]
          Length = 271

 Score =  197 bits (502), Expect = 1e-49
 Identities = 103/233 (44%), Positives = 141/233 (60%), Gaps = 5/233 (2%)

Query: 52  DKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNK--WPMGGSGVVEVPFLLS 109
           D D   I   ++       S  L+EGD+I  +    +N+  W      G+VEVP+ +S
Sbjct: 41  DPDDVDITTSILQSNNGSSEILMEGDLIVSNTRNAMKCWNNQCLWRKSSDGLVEVPYTVS 100

Query: 110 SKYDEPSHQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLA 169
           +++      + I  A+  F    TCIRFV     QRDFISI     GC+S +GR+GG QVVSLA
Sbjct: 101 NEFSYYHKKRIENAMKTFNTETCIRFVPRSSQRDFISIESRDGCYSYLGRTGGKQVVSLA 160

Query: 170 PT-CLQKGRGIVLHELMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSQSSNMLT 228
              C+     GI+ HEL H LGF+HEHTR+DRD Y+++NW  + P     NF     ++N+ T
Sbjct: 161 RYGCVY--HGIIQHELNHALGFYHEHTRSDRDEYVKINWENVAPHTIYNFQTQDTNNLNT 218

Query: 229 PYDYSSVMHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGC 281
           PYDY+S+MHYGR AFS  G+ TITP+  P+  IGQR ++S  DI R+ KLY C
Sbjct: 219 PYDYTSIMHYGRTAFSTNGMDTITPVPNPNQSIGQRRSMSRGDILRIKKLYSC 271
```

Tblastn vs. NCBI-est

```
Tissue - Uterus tumour

>gb|BI061462.1|BI061462 IL3-UT0117-070301-494-H12 UT0117 Homo sapiens cDNA.
          Length = 652

 Score =  175 bits (443), Expect = 2e-42
 Identities = 85/86 (98%), Positives = 85/86 (98%)
 Frame = -2

Query: 29  SCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLS 88
           SCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLS
Sbjct: 546 SCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLS 367

Query: 89  ATSNKWPMGGSGVVEVPFLLSSKYDE 114
           ATSNKWPMGGSGVVEVPFLLSSKY E
Sbjct: 366 ATSNKWPMGGSGVVEVPFLLSSKYGE 289
```

Figure 2: Human cDNA libraries

Library	Tissue/cell source	Vector	Host strain	Supplier	Cat. no.
1	human fetal brain	Zap II	XL1-Blue MRF'	Stratagene	936206
2	human ovary	GT10	LE392	Clontech	HL1098a
3	human pituitary	GT10	LE392	Clontech	HL1097a
4	human placenta	GT11	LE392	Clontech	HL1075b
5	human testis	GT11	LE392	Clontech	HL1010b
6	human substantia nigra	GT10	LE392	in house	
7	human fetal brain	GT10	LE392	in house	
8	human cortex brain	GT10	LE392	in house	
9	human colon	GT10	LE392	Clontech	HL1034a
10	human fetal brain	GT10	LE392	Clontech	HL1065a
11	human fetal lung	GT10	LE392	Clontech	HL1072a
12	human fetal kidney	GT10	LE392	Clontech	HL1071a
13	human fetal liver	GT10	LE392	Clontech	HL1064a
14	human bone marrow	GT10	LE392	Clontech	HL1058a
15	human peripheral blood monocytes	GT10	LE392	Clontech	HL1050a
16	human placenta	GT10	LE392	in house	
17	human SHSYSY	GT10	LE392	in house	
18	human U373 cell line	GT10	LE392	in house	
19	human CFPoc-1 cell line	Uni Zap	XL1-Blue MRF'	Stratagene	936206
20	human retina	GT10	LE392	Clontech	HL1132a

21	human urinary bladder	GT10	LE392	in house	
22	human platelets	Uni Zap	XL1-Blue MRF'	in house	
23	human neuroblastoma Kan + TS	GT10	LE392	in house	
24	human bronchial smooth muscle	GT10	LE392	in house	
25	human bronchial smooth muscle	GT10	LE392	in house	
26	human Thymus	GT10	LE392	Clontech	HL1127a
27	human spleen 5' stretch	GT11	LE392	Clontech	HL1134b
28	human peripheral blood monocytes	GT10	LE392	Clontech	HL1050a
29	human testis	GT10	LE392	Clontech	HL1065a
30	human fetal brain	GT10	LE392	Clontech	HL1065a
31	human substantia nigra	GT10	LE392	Clontech	HL1093a
32	human placenta#11	GT11	LE392	Clontech	HL1075b
33	human Fetal brain	GT10	LE392	Clontech	custom
34	human placenta #59	GT10	LE392	Clontech	HL5014a
35	human pituitary	GT10	LE392	Clontech	HL1097a
36	human pancreas #63	Uni Zap XR	XL1-Blue MRF'	Stratagene	937208
37	human placenta #19	GT11	LE392	Clontech	HL1008
38	human liver 5'stretch	GT11	LE392	Clontech	HL1115b
39	human uterus	Zap-CMV XR	XL1-Blue MRF'	Stratagene	980207
40	human kidney large-insert cDNA library	TriplEx2	XL1-Blue	Clontech	HL5507u

Figure 3: Predicted nucleotide sequence of INSP005 with translation

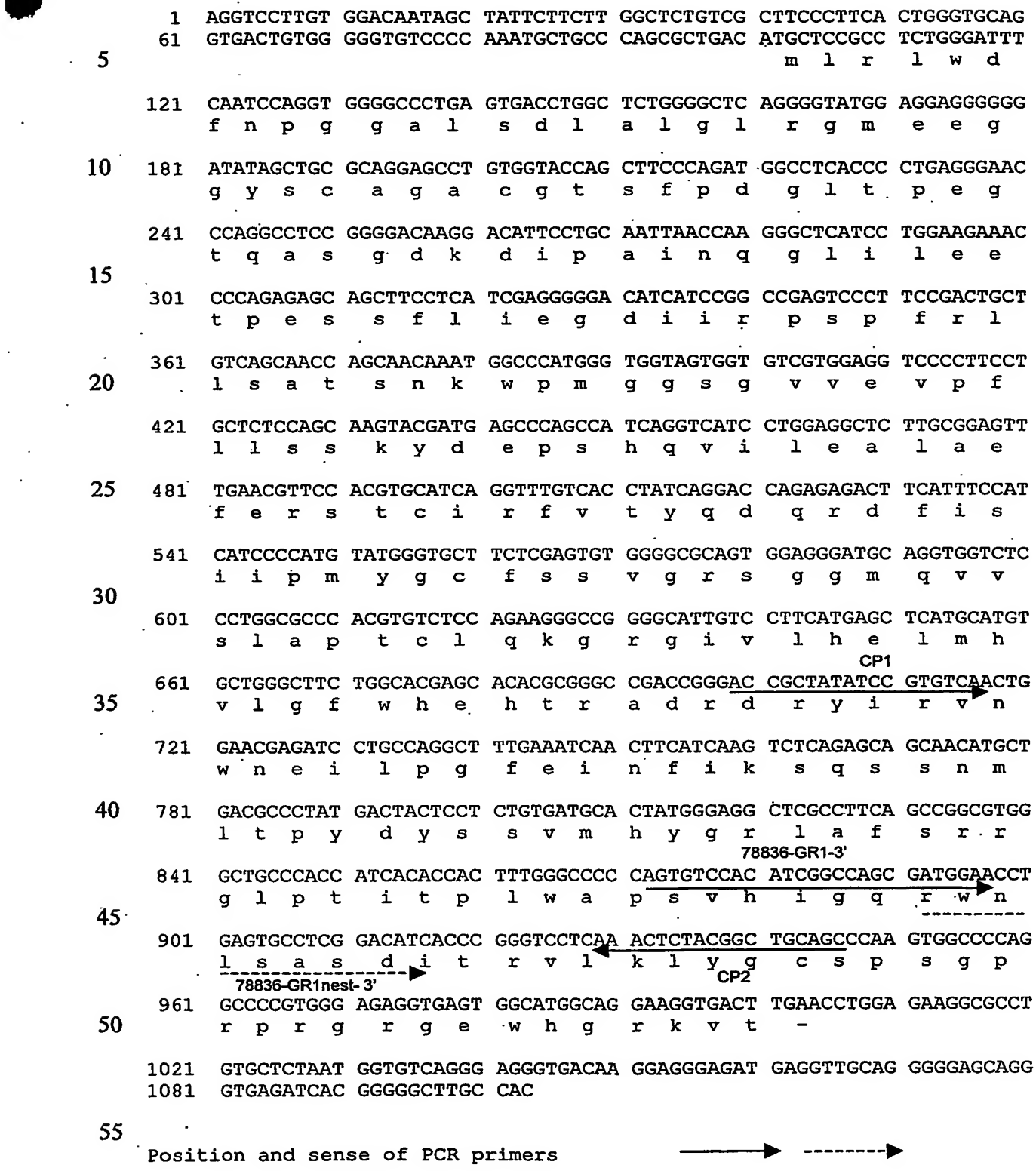

Figure 4: INSP005 Cloning primers

Primer	Name	Sequence (5'-3')
CP1	4C5	ACC GCT ATA TCC GTG TCA A
CP2	4C6	GCT GCA GCC GTA GAG TTT
GeneRacer 3'		GCT GTC AAC GAT ACG CTA CGT AAC G
78836-GR1-3'		AGT GTC CAC ATC GGC CAG CGA TGG AA
GeneRacer 3' nested		CGC TAC GTA ACG GCA TGA CAG TG
78836-GR1nest-3'		ATG GAA CCT GAG TGC CTC GGA CAT C
78836-FL-F	4C7	CTG TCA GCA ACC AGC AAC AA
78836-FL-R	9B2	AGC CAC AGG CTT AAT CTT CG
78836-FL2-F	9E6	TCT ACC ATG GAG GGT GTA GG

Figure 5: 3' Sequence of INSP005 identified by RACE PCR

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   1  ATGGAACCTG AGTGCCTCGG ACATCACCCG GGTCCTCAAA CTCTACGGCT GCAGCCCAAG
          w  n  l  s  a  s  d  i  t  r  v  l  k  l  y  g  c  s  p

  61  TGGCCCCAGG CCCCGTGGGA GAGGGTCCCA TGCCCACAGC ACTGGTAGGA GCCCCGCTCC
      s  g  p  r  p  r  g  r  g  s  h  a  h  s  t  g  r  s  p  a

 121  GGCCTCCCTA TCTCTGCAGC GGCTTTTGGA GGCACTGTCG GCGGAATCCA GGAGCCCCGA
      p  a  s  l  s  l  q  r  l  l  e  a  l  s  a  e  s  r  s  p

 181  CCCCAGTGGT TCCAGTGCGG GAGGCCAGCC CGTTCCTGCA GGGCCTGGGG AGAGCCCACA
      d  p  s  g  s  s  a  g  g  q  p  v  p  a  g  p  g  e  s  p

 241  TGGGTGGGAG TCCCCTGCCC TGAAAAAGCT CAGTGCAGAG GCCTCGGCAA GGCAGCCTCA
      h  g  w  e  s  p  a  l  k  k  l  s  a  e  a  s  a  r  q  p

 301  GACCCTAGCT TCCTCCCCAA GATCAAGGCC TGGAGCAGGT GCCCCCGGTG TTGCTCAGGA
      q  t  l  a  s  s  p  r  s  r  p  g  a  g  a  p  g  v  a  q

 361  GCAGTCCTGG CTGGCCGGAG TGTCCACCAA GCCCACAGTC CCATCTTCAG AAGCAGGAAT
      e  q  s  w  l  a  g  v  s  t  k  p  t  v  p  s  s  e  a  g

 421  CCAGCCAGTC CCTGTCCAGG GAAGCCCAGC TCTGCCAGGG GGCTGTGTAC CTAGAAATCA
      i  q  p  v  p  v  q  g  s  p  a  l  p  g  g  c  v  p  r  n

 481  TTTCAAGGGG ATGTCCGAAG ATTAAGCCTG TGGCTTCTGT CCCCAAGTAG GGAGGGCATC
      h  f  k  g  m  s  e  d

 541  CTCTGCCCAG TGGAGCTGGG TCGTCTACCT CTTGGCTCCT TTGGGCCACA CCACTGTCTT
 601  CCAGCCCCAA CCTACCACCC CATCTCAGAG GGCCAGGACT CTTCCCCTGT CTCTCTTCAC
 661  TGTGTTCCCC TAAGGGCTCC TAGGGCCAGG GGTTCTTCTA GCTCTGCCAC AGGGGAAGGC
 721  AGGCCTGGCT GTGCCTGCTC TTGACTTTTG CCCAGCCCTG GTGGATGCTG GGAATGGGAG
 781  GTGACATTCT CCAGGGACAG GTCCTGGAAG GGGTGGGGAA GAGGTAGGTT CCAGCCCCGC
 841  AGAACCCTGG AATCCCTCCT GTGCCTGAGG CCCTGCCCCC CAGCATGGAC TAATGGTGTC
 901  CCTACCTCTC CCTCAGGGCA GCCCTGTGGC TGGGACCCTG GGAACAGCCT CCCATCCCAC
 961  CCAACATGCC CAAGTGTGGG GGAATGTTCT ACAGCAGTGT AGCCTCCAGC CCTTCTCTCC
1021  AGGAGGCTTT GAGAGCCCAA CTTACTCCCC TGCAGAGCAG GAAGGTGGTA GGTCAAGTGT
1081  GGCCACCATT GGGGAGACGA GAAAGAAGTG GGGCCCCACC AGATTGCACA ATGGGAACCT
1141  CAGCTGGCCC CTGAACAGAG GACTCAGTTG TCTCCACCCT ACACCGCTAT TCCCTGGAGC
1201  TCAGCCAGGC GCAGCCTTGG AAGGAGAAAG GGCTGGGGTT ACCTGGCTTG TCCTCCTCCA
1261  GGAAAGCCCC CTTCCTCCTC TGCCCCAGCT CCCAGCCTGG CCTCCTCCAG GCAGGCCCTA
1321  CTCCTCTGCC CCAGCTCCGG CTTTCCCCAT GAGGTTTGTC CCAGGCATGA AGAAAGCATC
1381  CAGGGTGCCA ATGAGTGGGC CTAGGCCAGA GGCCCCTCAG TCCCCAAGGG TACTGTTTTG
1441  GTGGCCTTTC AGAGGGTCAA GGAAGCCCTG CTTGGGGTAG AAGGGGCAGG AGCCCCACAT
1501  GTTGGGGGAG GAAATAAAGT GGAGTGTGCT GTGCTGAAAA AAAAAAAAAA AAAA
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TAA Stop codon
AATAAA Consensus polyadenylation site

Figure 6: Sequencing primers

Primer	Sequence (5'-3')
T3	ATT AAC CCT CAC TAA AGG GA
T7	TAA TAC GAC TCA CTA TAG GG
SP6	ATT TAG GTG ACA CTA TAG

Figure 7: Putative full length INSP005a cloned from human uterus cDNA

```
   1  CTGTCAGCAA CCAGCAACAA ATGGCCCATG GGTGGTAGTG GTGTCGTGGA GGTCCCCTTC
                                m   g   g   s   g   v   v   e   v   p   f
      ---------->
      78836-FL-F
  61  CTGCTCTCCA GCAAGTACGA TGAGCCCAGC CGCCAGGTCA TCCTGGAGGC TCTTGCGGAG
        l   l   s   s   k   y   d   e   p   s   r   q   v   i   l   e   a   l   a   e
 121  TTTGAACGTT CCACGTGCAT CAGGTTTGTC ACCTATCAGG ACCAGAGAGA CTTCATTTCC
        f   e   r   s   t   c   i   r   f   v   t   y   q   d   q   r   d   f   i   s
 181  ATCATCCCCA TGTATGGGTG CTTCTCGAGT GTGGGGCGCA GTGGAGGGAT GCAGGTGGTC
        i   i   p   m   y   g   c   f   s   s   v   g   r   s   g   g   m   q   v   v
 241  TCCCTGGCGC CCACGTGTCT CCAGAAGGGC CGGGGCATTG TCCTTCATGA GCTCATGCAT
        s   l   a   p   t   c   l   q   k   g   r   g   i   v   l   h   e   l   m   h
 301  GTGCTGGGCT TCTGGCACGA GCACACGCGG GCCGACCGGG ACCGCTATAT CCGTGTCAAC
        v   l   g   f   w   h   e   h   t   r   a   d   r   d   r   y   i   r   v   n
 361  TGGAACGAGA TCCTGCCAGG CTTTGAAATC AACTTCATCA AGTCTCAGAG CAGCAACATG
        w   n   e   i   l   p   g   f   e   i   n   f   i   k   s   q   s   s   n   m
 421  CTGACGCCCT ATGACTACTC CTCTGTGATG CACTATGGGA GGCTCGCCTT CAGCCGGCGT
        l   t   p   y   d   y   s   s   v   m   h   y   g   r   l   a   f   s   r   r
 481  GGGCTGCCCA CCATCACACC ACTTTGGGCC CCCAGTGTCC ACATCGGCCA GCGATGGAAC
        g   l   p   t   i   t   p   l   w   a   p   s   v   h   i   g   q   r   w   n
 541  CTGAGTGCCT CGGACATCAC CCGGGTCCTC AAACTCTACG GCTGCAGCCC AAGTGGCCCC
        l   s   a   s   d   i   t   r   v   l   k   l   y   g   c   s   p   s   g   p
 601  AGGCCCCGTG GGAGAGGGTC CCATGCCCAC AGCACTGGTA GGAGCCCCGC CCCGGCCTCC
        r   p   r   g   r   g   s   h   a   h   s   t   g   r   s   p   a   p   a   s
 661  CTATCTCTGC AGCGGCTTTT GGAGGCACTG TCGGCGGAAT CCAGGAGCCC CGACCCCAGT
        l   s   l   q   r   l   l   e   a   l   s   a   e   s   r   s   p   d   p   s
 721  GGTTCCAGTG CGGGAGGCCA GCCCGTTCCT GCAGGGCCTG GGGAGAGCCC ACATGGGTGG
        g   s   s   a   g   g   q   p   v   p   a   g   p   g   e   s   p   h   g   w
 781  GAGTCCCCTG CCCTGAAAAA GCTCAGTGCA GAGGCCTCGG CAAGGCAGCC TCAGACCCTA
        e   s   p   a   l   k   k   l   s   a   e   a   s   a   r   q   p   q   t   l
 841  GCTTCCTCCC CAAGATCAAG GCCTGGAGCA GGTGCCCCCG GTGTTGCTCA GGAGCAGTCC
        a   s   s   p   r   s   r   p   g   a   g   a   p   g   v   a   q   e   q   s
 901  TGGCTGGCCG GAGTGTCCAC CAAGCCCACA GTCCCATCTT CAGAAGCAGG AATCCAGCCA
        w   l   a   g   v   s   t   k   p   t   v   p   s   s   e   a   g   i   q   p
 961  GTCCCTGTCC AGGGAAGCCC AGCTCTGCCA GGGGGCTGTG TACCTAGAAA TCATTTCAAG
        v   p   v   q   g   s   p   a   l   p   g   g   c   v   p   r   n   h   f   k
1021  GGGATGTCCG AAGATTAAGC CTGTGGCT
        g   m   s   e   d
      <----------
      78836-FL-R
```

Figure 8: INSP005a blastp vs. NCBI-nr

Top 10 hits:

```
Query= INSP005a
         (336 letters)

Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF
           1,247,039 sequences; 397,579,747 total letters

Searching.....................................................done
```

Sequences producing significant alignments:	Score (bits)	E Value
ref|XP_141346.1| similar to hatching enzyme EHE7 [Anguilla japon...	416	e-115
dbj|BAB68518.1| hatching enzyme EHE13 [Anguilla japonica]	187	2e-46
dbj|BAB68515.1| hatching enzyme EHE7 [Anguilla japonica]	186	4e-46
dbj|BAB68516.1| hatching enzyme EHE10 [Anguilla japonica]	186	4e-46
dbj|BAB68513.1| hatching enzyme EHE4 [Anguilla japonica]	186	5e-46
dbj|BAB68517.1| hatching enzyme EHE12 [Anguilla japonica]	183	3e-45
dbj|BAB68514.1| hatching enzyme EHE6 [Anguilla japonica]	183	3e-45
dbj|BAB68519.1| hatching enzyme EHE14 [Anguilla japonica]	182	7e-45
pir||C48826 high choriolytic hatching proteinase (EC 3.4.24.-) H...	171	1e-41
dbj|BAA12146.1| choriolysin H [Oryzias latipes]	171	2e-41

Top alignment to known metalloproteinase:

```
>dbj|BAB68518.1| hatching enzyme EHE13 [Anguilla japonica]
          Length = 271

 Score =  187 bits (475), Expect = 2e-46
 Identities = 93/183 (50%), Positives = 124/183 (66%), Gaps = 3/183 (1%)

Query: 5   GVVEVPFLLSSKYDEPSRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGR 64
           G+VEVP+ +SS++    ++ I  A+  F   TCIRFV    QRDFISI    GC+S +GR
Sbjct: 91  GLVEVPYTVSSEFSYYHKKRIENAMETFNTETCIRFVPRSSQRDFISIESRDGCYSYLGR 150

Query: 65  SGGMQVVSLAPT-CLQKGRGIVLHELMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINF 123
           +GG QVVSLA   C+    GI+ HEL H LGF+HEHTR+DRD Y+++NW  + P    NF
Sbjct: 151 TGGKQVVSLARYGCVY--HGIIQHELNHALGFYHEHTRSDRDEYVKINWENVAPHTIYNF 208

Query: 124 IKSQSSNMLTPYDYSSVMHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKL 183
            +  ++N+ TPYDY+S+MHYGR AFS  G+ TITP+  P+  IGQR ++S  DI R+ KL
Sbjct: 209 QEQDTNNLNTPYDYTSIMHYGRTAFSTNGMDTITPVPNPNQSIGQRRSMSKGDILRINKL 268

Query: 184 YGC 186
           Y C
Sbjct: 269 YSC 271
```

Figure 9: Map of PCR4-TOPO-IPAAAIPAAA7883-1

Molecule: pCR4 TOPO-IPAAA78836-1, 5005 bps DNA Circular
File Name: 13164.cm5, dated 24 Oct 2002

Description: Ligation of inverted 78836_F2/R8 PCR product into pCR4-TOPO linear vector*

Molecule Features:

Type	Start	End		Name	Description
REGION	205	221		M13	rev priming site
MARKER	243			T3	
REGION	262	294			Polylinker'
REGION	294	294			TOPO cloning site'
GENE	1315	308	C	IPAAA78836-1	
REGION	1342	295	C		Inserted PCR product
REGION	1343	1360			'Polylinker
REGION	1343	1343			'TOPO cloning site
MARKER	1395		C	T7	
REGION	1403	1418		M13	
GENE	2207	3001		KanR	
GENE	3205	4065		AmpR	
REGION	4210	4883		pUC ori	

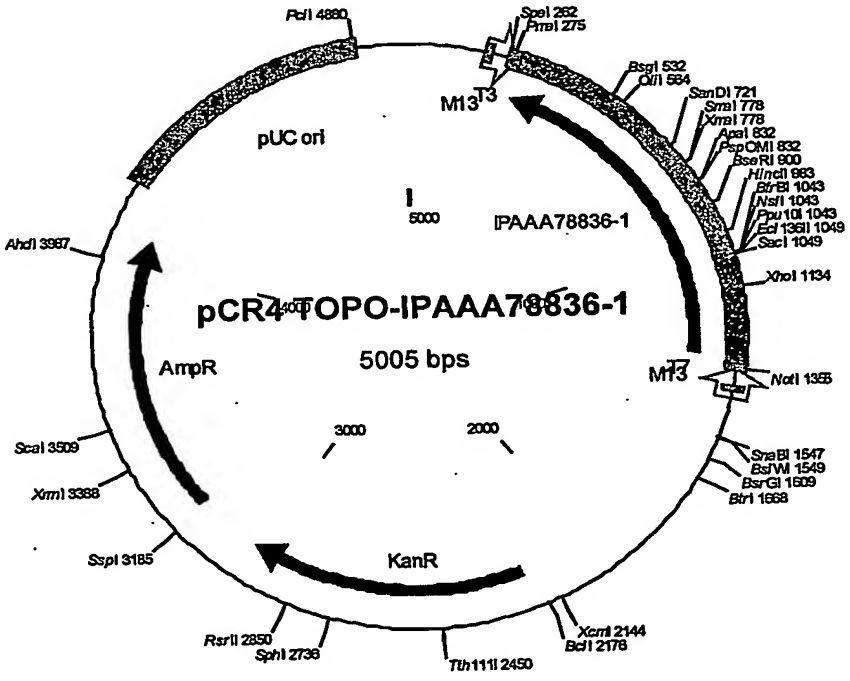


Figure 10: Putative full length INSP005b with alternative 5' end

```
                78836-FL2-F
1   TTCTA ------------------> TGTAG GGGGTCTCTG GCCTTGGGTG CTGGGTCTGC TCTCCTTGCC
              m  e  g  v  g  g  l  w  p  w  v  l  g  l  l  s  l

61  AGGTGTGATC CTAGGAGCGC CCCTGGCCTC CAGCTGCGCA GGAGCCTGTG GTACCAGCTT
    p  g  v  i  l  g  a  p  l  a  s  s  c  a  g  a  c  g  t  s

121 CCCAGATGGC CTCACCCCTG AGGGAACCCA GGCCTCCGGG GACAAGGACA TTCCTGCAAT
    f  p  d  g  l  t  p  e  g  t  q  a  s  g  d  k  d  i  p  a

181 TAACCAAGGG CTCATCCTGG AAGAAACCCC AGAGAGCAGC TTCCTCATCG AGGGGGACAT
    i  n  q  g  l  i  l  e  e  t  p  e  s  s  f  l  i  e  g  d

241 CATCCGGCCG AGTCCCTTCC GACTGCTGTC AGCAACCAGC AACAAATGGC CCATGGGTGG
    i  i  r  p  s  p  f  r  l  l  s  a  t  s  n  k  w  p  m  g

301 TAGTGGTGTC GTGGAGGTCC CCTTCCTGCT CTCCAGCAAG TACGATGAGC CCAGCCGCCA
    g  s  g  v  v  e  v  p  f  l  l  s  s  k  y  d  e  p  s  r

361 GGTCATCCTG GAGGCTCTTG CGGAGTTTGA ACGTTCCACG TGCATCAGGT TTGTCACCTA
    q  v  i  l  e  a  l  a  e  f  e  r  s  t  c  i  r  f  v  t

421 TCAGGACCAG AGAGACTTCA TTTCCATCAT CCCCATGTAT GGGTGCTTCT CGAGTGTGGG
    y  q  d  q  r  d  f  i  s  i  i  p  m  y  g  c  f  s  s  v

481 GCGCAGTGGA GGGATGCAGG TGGTCTCCCT GGCGCCCACG TGTCTCCAGA AGGGCCGGGG
    g  r  s  g  g  m  q  v  v  s  l  a  p  t  c  l  q  k  g  r

541 CATTGTCCTT CATGAGCTCA TGCATGTGCT GGGCTTCTGG CACGAGCACA CGCGGGCCGA
    g  i  v  l  h  e  l  m  h  v  l  g  f  w  h  e  h  t  r  a

601 CCGGGACCGC TATATCCGTG TCAACTGGAA CGAGATCCTG CCAGGCTTTG AAATCAACTT
    d  r  d  r  y  i  r  v  n  w  n  e  i  l  p  g  f  e  i  n

661 CATCAAGTCT CGGAGCAGCA ACATGCTGAC GCCCTATGAC TACTCCTCTG TGATGCACTA
    f  i  k  s  r  s  s  n  m  l  t  p  y  d  y  s  s  v  m  h

721 TGGGAGGCTC GCCTTCAGCC GGCGTGGGCT GCCCACCATC ACACCACTTT GGGCCCCCAG
    y  g  r  l  a  f  s  r  r  g  l  p  t  i  t  p  l  w  a  p

781 TGTCCACATC GGCCAGCGAT GGAACCTGAG TGCCTCGGAC ATCACCCGGG TCCTCAAACT
    s  v  h  i  g  q  r  w  n  l  s  a  s  d  i  t  r  v  l  k

841 CTACGGCTGC AGCCCAAGTG GCCCCAGGCC CCGTGGGAGA GGGTCCCATG CCCACAGCAC
    l  y  g  c  s  p  s  g  p  r  p  r  g  r  g  s  h  a  h  s

901 TGGTAGGAGC CCCGCTCCGG CCTCCCTATC TCTGCAGCGG CTTTTGGAGG CACTGTCGGC
    t  g  r  s  p  a  p  a  s  l  s  l  q  r  l  l  e  a  l  s

961 GGAATCCAGG AGCCCCGACC CCAGTGGTTC CAGTGCGGGA GGCCAGCCCG TTCCTGCAGG
    a  e  s  r  s  p  d  p  s  g  s  s  a  g  g  q  p  v  p  a

1021 GCCTGGGGAG AGCCCACATG GGTGGGAGTC CCCTGCCCTG AAAAAGCTCA GTGCAGAGGC
    g  p  g  e  s  p  h  g  w  e  s  p  a  l  k  k  l  s  a  e

1081 CTCGGCAAGG CAGCCTCAGA CCCTAGCTTC CTCCCCAAGA TCAAGGCCTG GAGCAGGTGC
    a  s  a  r  q  p  q  t  l  a  s  s  p  r  s  r  p  g  a  g
```

```
1141  CCCCGGTGTT GCTCAGGAGC AGTCCTGGCT GGCCGGAGTG TCCACCAAGC CCACAGTCCC
        a  p  g  v  a  q  e  q  s  w  l  a  g  v  s  t  k  p  t  v

1201  ATCTTCAGAA GCAGGAATCC AGCCAGTCCC TGTCCAGGGA AGCCCAGCTC TGCCAGGGGG
        p  s  s  e  a  g  i  q  p  v  p  v  q  g  s  p  a  l  p  g

1261  CTGTGTACCT AGAAATCATT TCAAGGGGAT GTCCGAAGAT TAAGCCTGTG GCT
        g  c  v  p  r  n  h  f  k  g  m  s  e  d
```

78836-FL-R

Figure 11: INSP005b blastp vs. NCBI-nr:

Top 10 hits:

```
Query= INSP005b
         (431 letters)

Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF
           1,247,039 sequences; 397,579,747 total letters

Searching.....................................................done

                                                                  Score     E
Sequences producing significant alignments:                       (bits)  Value

ref|XP_141346.1| similar to hatching enzyme EHE7 [Anguilla japon...   540  e-152
dbj|BAB68513.1| hatching enzyme EHE4 [Anguilla japonica]             198  1e-49
dbj|BAB68518.1| hatching enzyme EHE13 [Anguilla japonica]            198  1e-49
dbj|BAB68516.1| hatching enzyme EHE10 [Anguilla japonica]            197  3e-49
dbj|BAB68515.1| hatching enzyme EHE7 [Anguilla japonica]             196  4e-49
dbj|BAB68514.1| hatching enzyme EHE6 [Anguilla japonica]             196  7e-49
dbj|BAB68517.1| hatching enzyme EHE12 [Anguilla japonica]            194  3e-48
dbj|BAB68519.1| hatching enzyme EHE14 [Anguilla japonica]            191  1e-47
pir||C48826 high choriolytic hatching proteinase (EC 3.4.24.-) H...   187  3e-46
dbj|BAA12146.1| choriolysin H [Oryzias latipes]                      186  4e-46
```

Top alignment to known metalloproteinase:

```
>dbj|BAB68518.1| hatching enzyme EHE13 [Anguilla japonica]
           Length = 271

 Score =  198 bits (503), Expect = 1e-49
 Identities = 103/233 (44%), Positives = 144/233 (61%), Gaps = 5/233 (2%)

Query: 52  DKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNK--WPMGGSGVVEVPFLLS 109
           D D   I   ++       S  L+EGD++  +    ++  +N+  W     G+VEVP+ +S
Sbjct: 41  DPDDLDITARILQSNNGSSEILMEGDMVVSNTRNAINCWNNQCLWRKSSDGLVEVPYTVS 100

Query: 110 SKYDEPSRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLA 169
           S++    ++ I  A+  F   TCIRFV    QRDFISI    GC+S +GR+GG QVVSLA
Sbjct: 101 SEFSYYHKKRIENAMETFNTETCIRFVPRSSQRDFISIESRDGCYSYLGRTGGKQVVSLA 160
```

```
Query: 170 PT-CLQKGRGIVLHELMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSRSSNMLT 228
             C+    GI+ HEL H LGF+HEHTR+DRD Y+++NW  + P    NF +  ++N+ T
Sbjct: 161 RYGCVY--HGIIQHELNHALGFYHEHTRSDRDEYVKINWENVAPHTIYNFQEQDTNNLNT 218

Query: 229 PYDYSSVMHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGC 281
           PYDY+S+MHYGR AFS  G+ TITP+  P+  IGQR ++S  DI R+ KLY C
Sbjct: 219 PYDYTSIMHYGRTAFSTNGMDTITPVPNPNQSIGQRRSMSKGDILRINKLYSC.
```

Figure 12: Map of PCR-TOPO-IPAAA78836-2

Molecule: pCR4 TOPO-IPAAA78836-2, 5269 bps DNA Circular
File Name: 13296.cm5, dated 24 Oct 2002

Description: Ligation of inverted IPAAA78836v2 into pCR4-TOPO linear vector*

Molecule Features:

Type	Start	End		Name	Description
REGION	205	221		M13	rev priming site
MARKER	243			T3	
REGION	262	294			Polylinker'
REGION	294	294			TOPO cloning site'
GENE	1600	307	C	IPAAA78836-2	
REGION	1607	1624			'Polylinker
REGION	1607	1607			'TOPO cloning site
MARKER	1659		C	T7	
REGION	1667	1682		M13	
GENE	2471	3265		KanR	
GENE	3469	4329		AmpR	
REGION	4474	5147		pUC ori	

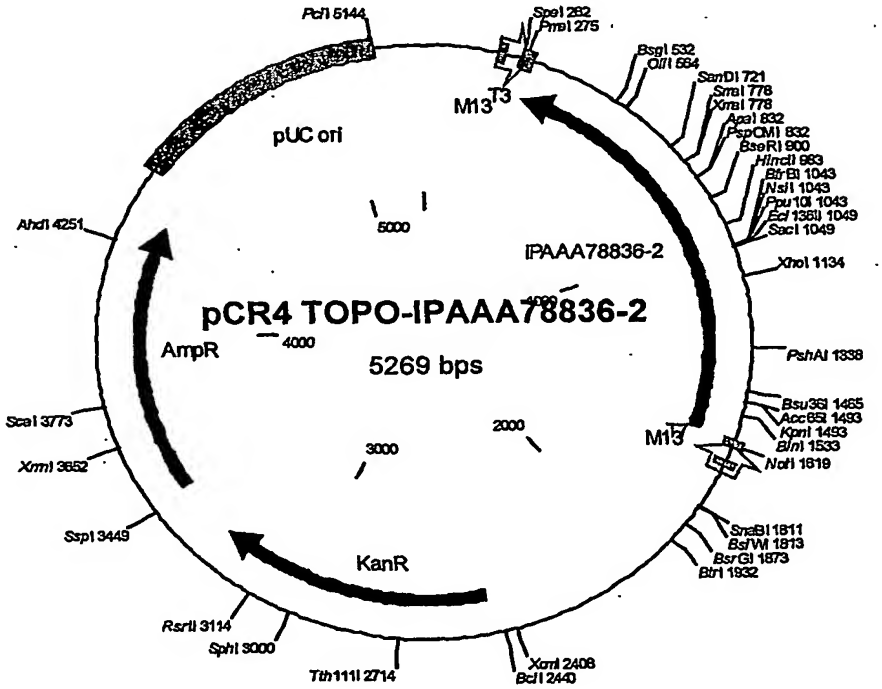


Figure 13: Multiple alignment of the INSP005 predicted sequence, the INSP005a sequence, the INSP005b sequence and prior art sequences of interest

Active site residues are highlighted in grey below.

```
WO2002/16566-A2      -----MEGVGGLWPWVLGLLSLPGVILGAPLASSCAGACGTSFPDGLTPEGTQASGDKDI
AX526191             MSCCLVSPVGAPGICVCPCLSGPGVILGAPLASSCAGACGTSFPDGLTPEGTQASGDKDI
INSP005 PREDICTION   ------------------------------------------------------------
INSP005b             -----MEGVGGLWPWVLGLLSLPGVILGAPLASSCAGACGTSFPDGLTPEGTQASGDKDI
INSP005a             ------------------------------------------------------------

WO2002/16566-A2       PAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNKWPMGGSGVVEVPFLLSSKYDEP
AX526191              PAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNKWPMGGSGVVEVPFLLSSKYDEP
INSP005 PREDICTION    ------------------------------------WPMGGSGVVEVPFLLSSKYDEP
INSP005b              PAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNKWPMGGSGVVEVPFLLSSKYDEP
INSP005a              ------------------------------------MGGSGVVEVPFLLSSKYDEP
                                                           ********************

WO2002/16566-A2      SRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLAPTCLQK
AX526191             SRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLAPTCLQK
INSP005 PREDICTION   SHQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLAPTCLQK
INSP005b             SRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLAPTCLQK
INSP005a             SRQVILEALAEFERSTCIRFVTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLAPTCLQK
                     *:************************************************************

WO2002/16566-A2      GRGIVLHEIMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSRSSNMLTPYDYSSV
AX526191             GRGIVLHEIMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSRSSNMLTPYDYSSV
INSP005 PREDICTION   GRGIVLHEIMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSQSSNMLTPYDYSSV
INSP005b             GRGIVLHEIMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSRSSNMLTPYDYSSV
INSP005a             GRGIVLHEIMHVLGFWHEHTRADRDRYIRVNWNEILPGFEINFIKSQSSNMLTPYDYSSV
                     ************************************************:*************

WO2002/16566-A2      MHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGCSPSGPRPRGRG---
AX526191             MHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGCSPSGPRPRGRGSHA
INSP005 PREDICTION   MHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGC--------------
INSP005b             MHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGCSPSGPRPRGRGSHA
INSP005a             MHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGCSPSGPRPRGRGSHA
                     ************************************************:.:.. . . .: :
```

```
WO2002/16566-A2  ----------------------------------------------EWHG---RKVT
AX526191         HSTGRSPAPASLSLQRLLEALSAESRSPDPSGSSAGGQPVPAGPGESPHGWESPALKKLS
INSP005 PREDICTION ----------------------------------------------------------
INSP005b         HSTGRSPAPASLSLQRLLEALSAESRSPDPSGSSAGGQPVPAGPGESPHGWESPALKKLS
INSP005a         HSTGRSPAPASLSLQRLLEALSAESRSPDPSGSSAGGQPVPAGPGESPHGWESPALKKLS
                  ::. :.:.:: : .   .: ::.: :...:.:::.... .:....:.    ..:  . :

WO2002/16566-A2  ----------------------------------------------------------
AX526191         AEASARQPQTLASSPRSRPGAGAPGVAQEQSWLAGVSTKPTVPSSEAGIQPVPVQGSPAL
INSP005 PREDICTION ----------------------------------------------------------
INSP005b         AEASARQPQTLASSPRSRPGAGAPGVAQEQSWLAGVSTKPTVPSSEAGIQPVPVQGSPAL
INSP005a         AEASARQPQTLASSPRSRPGAGAPGVAQEQSWLAGVSTKPTVPSSEAGIQPVPVQGSPAL
                  :.::: ...: :::. : ..:.:.. :...:  :. ::..: .::.:. .. . ...:.:

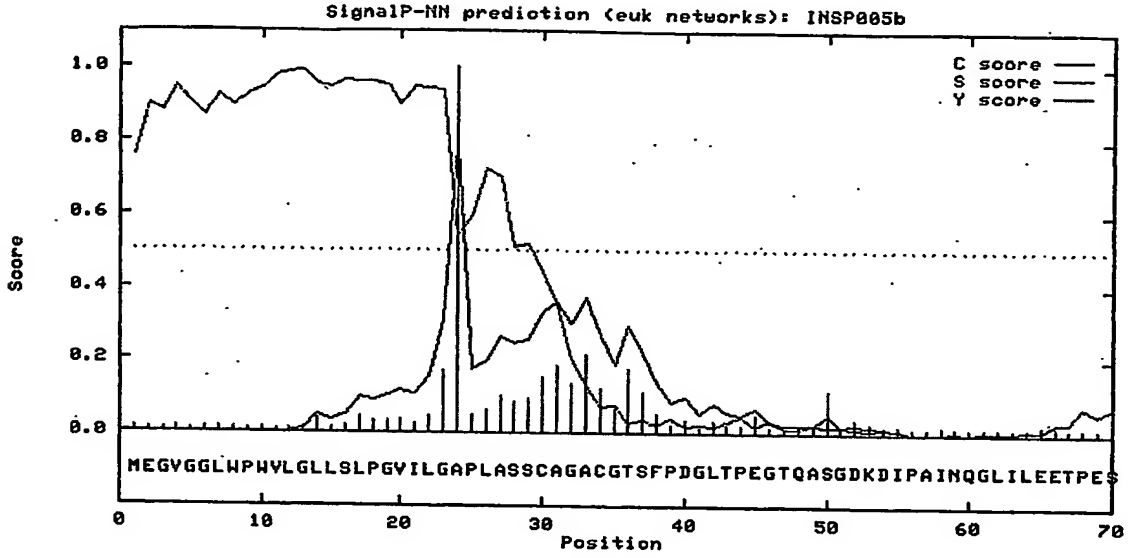
WO2002/16566-A2  ----------------
AX526191         PGGCVPRNHFKGMSED
INSP005 PREDICTION ----------------
INSP005b         PGGCVPRNHFKGMSED
INSP005a         PGGCVPRNHFKGMSED
                  .... . .  .. :..
```

Figure 14: INSP005b Predicted Signal Peptide

Signal P predictions were made on the basis of the INSP005b polypeptide sequence using neural networks (NN) and hidden Markov models (HMM) trained on eukaryotes:

>INSP005b

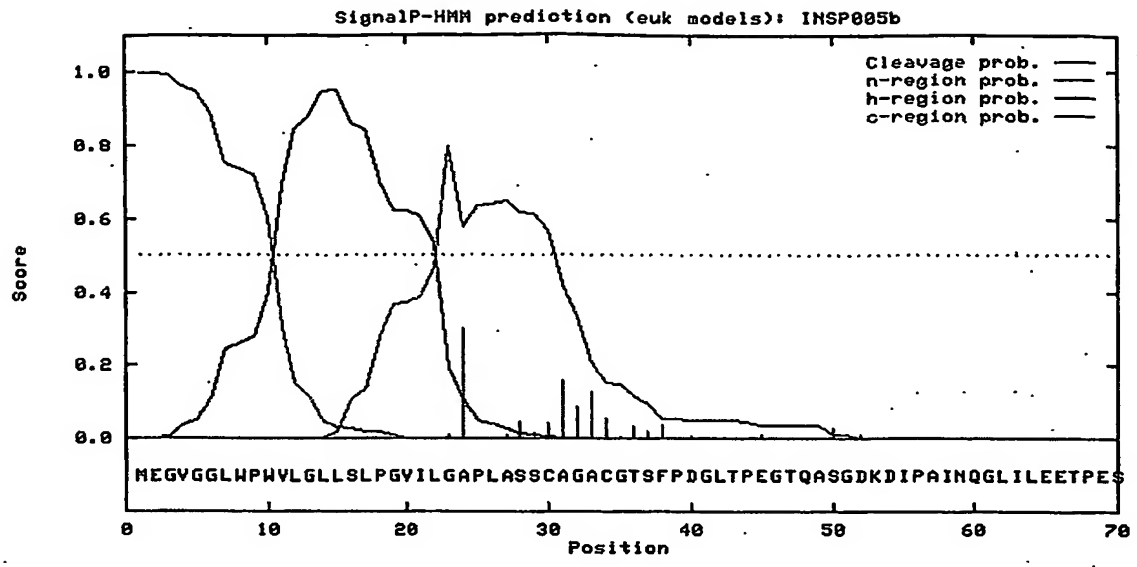
SignalP-NN result:



data

```
>INSP005b                 length = 70
# Measure  Position  Value   Cutoff  signal peptide?
  max. C     24       1.000   0.33    YES
  max. Y     24       0.789   0.32    YES
  max. S     13       0.991   0.82    YES
  mean S      1-23    0.929   0.47    YES
# Most likely cleavage site between pos. 23 and 24: ILG-AP
```

SignalP-HMM result:



data

```
>INSP005b
Prediction: Signal peptide
Signal peptide probability: 0.996
Signal anchor probability: 0.003
Max cleavage site probability: 0.302 between pos. 23 and 24
```